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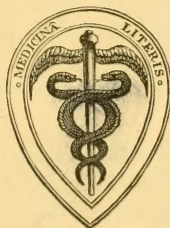
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Studies on the Comparative Anatomy of  
Sponges.

III.—On the Anatomy of *Grantia labyrinthica*, Carter, and the so-called Family *Teichonidæ*.

By

**Arthur Dendy, M.Sc., F.L.S.,**

Demonstrator and Assistant Lecturer in Biology and Fellow of Queen's  
College in the University of Melbourne.

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With Plates I—IV.

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INTRODUCTION.

DURING the past few years Mr. J. Bracebridge Wilson has, by perseveringly dredging in the neighbourhood of Port Phillip, accumulated a collection of sponges which already numbers something like 2000 specimens, and is probably the most complete collection ever brought together from the shores of any one country. The entire collection has been generously placed in my hands for investigation, and Professor McCoy has likewise kindly placed at my disposal the collection contained in the National Museum at Melbourne. The task of dealing with so large a mass of material is, I need hardly say, one of great magnitude, and the systematic investigation must necessarily extend over several years. The difficulty of the work, so far as identification of species is concerned, has been greatly lessened by the courtesy of Mr. H. J. Carter, who has generously sent me his own copy of his work on the Port Phillip *Calcispongiæ* (1), containing a large number of unpublished sketches; and of Dr. Günther, to whom I am deeply indebted

for a large series of duplicate pieces of named sponges from the British Museum collection, most kindly sent to me since I left England.

It is hoped that the final account of the collection will be embodied in one of a series of reports on the marine zoology of Port Phillip, which it is intended to publish under the auspices of the Port Phillip Exploration Committee of the Royal Society of Victoria. Meanwhile, even during the preliminary arrangement of the collection, forms are constantly being met with which deserve special anatomical investigation. One such I have already described in the present series of studies (2); and I hope, as time permits, to be able to deal similarly with a number of others.

The more one studies the group, the more is one convinced of the necessity of thorough and minute anatomical investigation as a basis for classification. Especially in the present transitional state of our knowledge of the sponges anatomical investigation must precede systematic work; and the greater the number of types selected for such investigation, the greater will be the value of the scheme of classification ultimately arrived at. Polymorphism and homoplasy occur so generally and to such an extraordinary degree amongst the Porifera, that true genetic relationships can be determined only by most careful examination of the internal anatomy, and especially of the skeleton and canal system, although even these systems are by no means exempt from the general rule.

The sponge which forms the principal subject of the present contribution is a large and singularly beautiful calcisponge, originally described (3) by Mr. Carter under the name *Teichonella labyrinthica*. As Mr. Carter subsequently discovered, the sponge is very far removed from the genus *Teichonella*, and must be placed amongst the Sycons, where for the present, at any rate, it may be classed in the genus *Grantia*.<sup>1</sup>

The material at my disposal for investigating this sponge consisted principally of a number of fine adult specimens col-

<sup>1</sup> Vide Vosmaer's diagnosis of this (20).

lected by Mr. Wilson, and preserved in ordinary methylated spirit. This was supplemented by some fragments taken from a fresh specimen by myself when out dredging with Mr. Wilson, and preserved in absolute alcohol, and by three young examples of great interest. Sections were cut in different directions by hand, by the freezing microtome, and by the paraffin method. For studying the skeleton, hand-cut sections of unstained material and preparations of the spicules boiled out with caustic potash are most serviceable. For minute anatomical and histological work, thinner sections of material stained with borax carmine, and cut by the ordinary paraffin method, were found to yield good results.

### The Anatomy of *Grantia labyrinthica*.

#### (a) Historical.

The sponge under consideration was, as I have stated above, originally described by Mr. Carter in the 'Annals and Magazine of Natural History' in 1878 (3), apparently from a dry and imperfect specimen in the British Museum collection. The brief description is confined almost entirely to the skeleton and the external characters, but the author observes that "in spiculation and in the structure of the lamina it is closely allied to *Grantia compressa*, Fleming." He refers the sponge, however, as already noted, to his genus *Teichonella*.

In 1886 Mr. Carter was able (1) to supplement his original description from the examination of specimens dredged and sent to England by Mr. Bracebridge Wilson. He observes that "the sponge is goblet-shaped in general form, and not simply 'vallate,' like *T. prolifera*; also that a quadri-radiate forms part of its spiculation; hence these additional facts render it necessary that it should be relegated to the vicinity of *Grantia compressa*, where its generic name might be changed from '*Teichonella*' to '*Grantia*.'"

Previously to this date, however, Mr. Carter had published in the 'Annals and Magazine of Natural History' a remark-



able paper (4) on the "Mode of Circulation in the Spongida," in which he advocates the theory that "the particles that are taken in with the water through the pores of the dermis fall directly into the subdermal cavities, and pass thence into the large excretory canals, from which they are afterwards deflected to their destination through smaller branches, whose apertures may be seen in the walls of the former." He adds, "This perhaps is best seen in *Teichonella labyrinthica*, wherein the chambers, which are arranged in juxtaposition perpendicularly to the lamina of which the sponge is composed, thus pass directly through it from one side to the other, having therefore on one side the pores or pore-dermis, and on the other the vent; in short, exactly like those of *Grantia compressa*, only there is no cloaca. We must, however, regard this chamber as at once ampullaceous sac and excretory canal; for the pore-dermis being at one end or side of the lamina and the vent at the other, the circulation passes into the former and out at the latter, through the chamber, where the nutritive particles are instantly taken up by the spongozoa lining its cavity. Hence the holes in the walls of the chamber, which are very numerous, may serve for the purpose of intercommunication, where the walls of the neighbouring chambers are in direct contact with each other, or for the purpose of allowing the ova developed in the intercameral tissue to pass into the chamber and thus be expelled. Therefore these holes would seem to have more functions than those ascribed to them in the wall of the ampullaceous sac of the so-called 'siliceous sponges,' ex. gr. *Spongelia avara*." For a further elucidation of Mr. Carter's views on the canal system of sponges in general, the student is referred to his paper (5) "On the Position of the Ampullaceous Sac and the Function of the Water Canal System in the Spongida;" at present I wish to consider only the particular case of *Grantia labyrinthica*. Mr. Carter's account of the arrangement of the canal system in this sponge is supplemented by a figure (Pl. IV, fig. 7), wherein the flagellated chamber (= ampullaceous sac or radial tube) is represented as being perfectly

straight and cylindrical, with a pore-sieve opening into it at one end and a vent at the other.

Such, then, was the state of our knowledge of *Grantia labyrinthica*, one of the most remarkable of all the calcareous sponges, when I commenced my investigations. How far my own observations agree or disagree with Mr. Carter's descriptions will appear subsequently.

(b) External Characters.

*Grantia labyrinthica* is very large for a calcareous sponge, well-grown specimens being about three inches in height and a little more in breadth, so that it is probably the largest of all the Sycons. Hence it is peculiarly well adapted for anatomical investigation.

A better idea of the external appearance will perhaps be gathered from an examination of fig. 4 than from any description which I can give. It will be seen that the adult sponge consists essentially of a thin-walled cup or basin, with a widely open mouth showing no signs of constriction, and thus differing markedly from the oscula of all other known Sycons. The wall of the cup, however, which, as I shall show later on, is in the young sponge simple and not folded, in the adult becomes greatly convoluted and folded upon itself, without, however, ever losing its character of a single continuous lamella. Thus, while in most Sycon sponges the circumferential growth of the tube or cup after a time diminishes as the sponge grows older, giving rise to a more or less constricted osculum, in *Grantia labyrinthica* precisely the reverse takes place, and the circumferential growth so far outstrips the vertical growth that the sponge wall becomes thrown into numerous deep folds, while the osculum becomes enormously wide and bounded by a deeply sinuous margin. The cup thus formed is attached by the middle of its lower surface to a stout cylindrical stalk, which is also a subsequent development not present in the very young sponge. At its lower end the stalk is fixed to the rock or other body upon which the free swimming embryo may have chanced to come to rest.

This peculiar external form is to some extent paralleled by certain species of the genus *Phyllospongia* amongst the horny sponges, as will be evident on referring to Lendenfeld's figures (6). By far the most remarkable parallelism, however, is exhibited by a *Renieriæ* species, not yet determined, which so closely resembles a young *Grantia labyrinthica* in external appearance that I at first placed it along with other specimens of that sponge to be figured, and only found out my mistake on microscopical examination. This interesting example of homoplasia serves well to show the necessity of microscopical examination before even the approximate position of any particular sponge can be safely determined.

Both surfaces of the cup are smooth, and the inner surface is at once seen to be perforated by innumerable minute and closely placed apertures, the exhalant openings of the flagellated chambers. These give to the surface a minutely punctate appearance, which is absent immediately below the free margin, where the sponge wall becomes very thin and translucent. On the outer surface of the cup the pore-sieves form less obvious markings.

(c) The skeleton.

The Spicules.—The calcareous spicules composing the skeleton of the sponge are of three main types—triradiate, quadriradiate, and uniaxial (oxeote). Each of these types occurs in the sponge under more than one modification, according to its position.

Triradiate Spicules.

These form the main mass of the skeleton. The different modifications which they present in different parts of the sponge depend chiefly upon the relative length of the rays; and, to some extent, upon the proportions of the angles between them. Thus we find a more or less gradual series between approximately equiradiate and equiangular spicules on the one hand, in which all three rays are of about the same length, and the angles between them nearly equal (fig.



16), and extremely inequiradiate and inequangular spicules, of the form termed by Haeckel (7) "sagittal," on the other. In the sagittal spicules two rays are about equal in length, while the third has either grown out into a very long and slender shaft, which may attain to three or four times the length of either of the others (figs. 13, 14), or (much more rarely) remained short while the other two have grown long (fig. 18). The angle between the two paired arms, or the oral angle, as Haeckel terms it, is greater than either of the other two angles, which are equal.

Figs. 13 to 19 represent seven triradiate spicules, and illustrate the variation in the proportions of the rays and angles. The commonest form is the sagittal, with one ray (the shaft) much longer than the other two. It is important to notice that, as a general rule at any rate, the three rays of the triradiate do not all lie in exactly the same plane, so that if the spicule were laid down upon an even surface it would rest upon the ends of the three rays, with the centre elevated. This fact is not shown in the figures, which are merely outlines drawn with the camera. In the sagittal spicules the shaft is usually perfectly straight, but often of the beautiful spear-like form shown in figs. 13 and 14. The two paired rays, on the other hand, are often slightly curved.

The triradiates vary considerably in size. The following measurements are taken from a well-grown spicule, of the form shown in fig. 13, and all the other spicules (from figs. 7 to 20 inclusive) are drawn to the same scale.

Length of the shaft 0.38 mm.

Length of the paired rays 0.12 mm.

### Quadriradiate Spicules.

These are probably to be regarded only as further modifications of the fundamental triradiate type, for although they are quadriradiate they are still only triaxial. Their shape is usually that represented in fig. 20. It will be seen that the third ray of a normal triradiate spicule has become much

shortened, while an additional fourth ray has appeared as a direct continuation of the third ray in the angle between the two paired rays. The cessation of growth of the third ray in its usual direction is perhaps to be accounted for by the outgrowth of the new fourth ray in the exactly opposite direction. The additional fourth ray is usually slightly hastate in form, the surface of the spear-head being at the same time slightly roughened. Occasionally, as shown in fig. 21, this peculiarity in form is very much more strongly marked. The size of the quadriradiates may be estimated from fig. 20.

### Uniaxial Spicules.

The shape of these spicules is a modification of the oxeote type, in which one end is markedly broader than the other, and often decidedly hastate (figs. 10, 11). Fig. 11 represents a spicule of the more usual size and form; figs. 7, 8, 9 represent giant modifications of the same from the margin of the osculum, and fig. 12 represents the large-sized form found in the stem. All the figures are drawn to the same scale as the triradiates.

**The Arrangement of the Skeleton.**—In dealing with this part of my subject I propose to follow the plan laid down by Haeckel in his 'Monograph of the Calcareous Sponges,' which seems to be in all respects the most satisfactory.

In the Sycons Haeckel distinguishes the following skeletal systems, which, for the sake of convenience, I arrange in an order slightly different from his:

1. The dermal skeleton, protecting the outer or dermal surface of the tube or cup, of which the Sycon individual consists.
2. The gastral skeleton, protecting the inner or gastral surface.
3. The skeleton of the peristome, protecting the margin of the osculum.
4. The tubar skeleton, lying between the dermal and gastral systems, and affording support to the flagellated chambers.

5. The skeleton of the base or stalk (where one is present).

It will be evident from the following account that *Grantia labyrinthica* conforms very closely in the arrangement of the skeleton to the normal *Sycon* plan.

### The Dermal Skeleton.

This forms a distinct cortex of somewhat varying thickness over the outer surface of the sponge (figs. 22, 25). The main mass of this cortex consists of large sagittal triradiates, arranged so that the long unpaired ray points towards the base of the sponge. Even within the limits of the dermal cortex the triradiates exhibit considerable individual variation in shape and size. In the inner portion of the cortex numbers of the singularly beautiful sagittal triradiates represented in figs. 13 and 14 occur.

The outermost portion of the dermal skeleton consists of great numbers of small oxete spicules of the form represented in figs. 10 and 11, placed at right angles to the surface, with their narrow ends embedded amongst the large triradiates, and their broad ends projecting freely (fig. 22). In the pore-areas the dermal skeleton is practically reduced to this outer layer of small oxea, many of which appear to be quite flat in the thin dermal membrane.

### The Gastral Skeleton.

The gastral skeleton (figs. 22, 25) forms a protective cortex over the inner surface of the cup, exactly as the dermal skeleton does over the outer. The gastral and dermal cortex do not differ very greatly in thickness, although in this respect there seems to be a good deal of variation. In the specimen before me as I write the gastral cortex is decidedly thinner than the dermal. Like the latter it is made up principally of triradiate spicules interwoven to form a feltwork; as before, the spicules are usually sagittal, with the long arm pointing towards the base of the sponge. The beautiful



sagittal triradiates represented in figs. 13 and 14 occur also in the gastral cortex; and here, too, we meet with the quadriradiate spicules. The latter surround the short exhalant canals of the flagellated chambers, being arranged as shown in figs. 21 and 24, with the additional fourth ray projecting towards or into the lumen of the canal. Occasionally an unusually small exhalant canal is met with, cut transversely, in tangential sections of the cup-wall, a little below the level of the ordinary exhalant canals. Such small canals (fig. 21) are surrounded by quadriradiate spicules of the slightly different form already described.

On the extreme outside of the gastral cortex (i. e. the extreme inside of the cup-wall) there occur abundant oxecote spicules like those of the dermal skeleton, and arranged, in close-set tufts, perpendicularly to the surface with their broad ends outwards (fig. 22). Mr. Carter (3) states that the linear (oxecote) spicules on the "vent-side" of the cup-wall are twice the length of those on the "pore-side." This does not hold good as a general rule, for in the specimen before me the reverse is the case (fig. 22).

### The Skeleton of the Peristome.

This consists of a fringe of the giant oxecote spicules already described (figs. 7—9), arranged with their broad ends projecting freely around the margin of the osculum, and their narrow ends embedded amongst a mass of approximately equiradiate triradiates, into which the dermal and gastral skeletons merge. The oscular fringe (fig. 25, *o. sp.*) thus formed is so very slightly developed in proportion to the size of the whole sponge that it is scarcely noticeable with the naked eye.

### The Tubar Skeleton.

According to the manner in which the spicules are arranged around the flagellated chambers (radial tubes) Haeckel (7) distinguishes two types of tubar skeleton, (1) articulate (*gegliedertes*) and (2) inarticulate (*ungegliedertes*). The articulate

tubar skeleton is always composed of triradiates, and is distinguished by the fact that along the length of the chamber there are always two or more transverse zones or "joints" of triradiates, one behind the other. The triradiates are usually sagittal, and the shaft is directed towards the dermal surface. The longer the flagellated chamber the greater is the number of joints in its skeleton.

Haeckel adds that in most Sycons with an articulate tubar skeleton the separate joints of the latter become specially differentiated. Thus the first or innermost joint is longer than the following; the outermost, on the other hand, is the shortest. The triradiates of the first joint, again, are most markedly sagittal, while their basal ray is unusually elongated, and their paired lateral rays are placed with convex, curved margin beneath the gastral surface. The triradiates of the following joints are usually less markedly sagittal, their basal ray less hypertrophied, and their oral angle generally smaller. Finally, at the distal end of the tube, towards the dermal surface, the sagittal triradiates generally pass over into the regular or subregular, and often into the irregular form.

The tubar skeleton in *Grantia labyrinthica* (fig. 22) is articulate, and agrees very exactly with the general description of such a skeleton given by Haeckel. The first joint is much longer than any of the others, and the sagittal triradiates composing it (=subgastral triradiates of Haeckel) are modified precisely as he describes, their short, curved, lateral arms lying beneath, or, indeed, forming a portion of, the gastral skeleton. The number of joints depends upon the length of the flagellated chamber, and this again varies with its position in the sponge, the older and longer chambers being situate nearer the base, and the younger and shorter ones nearer the margin of the cup.

It is hoped that fig. 22, which is drawn on the same plan as that adopted by Haeckel, will make all these points clear without further description.

It remains to be added that the two shorter arms of each triradiate curve slightly towards one another, so as partly to

embrace the chamber which they help to support. This is seen in figs. 28 and 29, where, owing to the direction of the section at right angles to the long axes of the flagellated chambers, the shafts of the triradiates are cut off, while the paired lateral arms are seen partially surrounding the chambers.

### The Skeleton of the Stalk.

This consists essentially of a confused mass of closely interwoven sagittal triradiates with very long and slender arms. One of these spicules is represented in fig. 19; often they are more or less irregular in form. On the extreme outside there is a layer of oxete spicules disposed at right angles to the surface. Most of these spicules are small, and very like those found over the surfaces of the cup, but a large number are modified into giant forms (fig. 12), differing somewhat in shape from those which form the oscular fringe. These giant spicules give to the surface of the stalk a more or less hispid character. They are remarkable from the fact that they project to an unusual extent, so that commonly less than a quarter of the length of the spicule is embedded in the tissues of the sponge. The outer ends of the spicules are consequently generally worn or broken.

#### (d) The canal system.

*Grantia labyrinthica* appears to agree more closely with respect to the arrangement of the canal system with Haeckel's *Sycortis lævigata* (7) than with any other described form. The canal system of all the Sycons is, of course, fundamentally the same, but numerous, by no means insignificant, variations occur, especially with regard to the inhalant pores and canals.

The canal system of calcareous sponges may be described in precisely the same terms as that of the siliceous and horny sponges, and since it is advisable to preserve uniformity of nomenclature wherever possible, I shall follow Poléjaeff's example (8) in making use of such general terms as "flagellated chamber" and "inhalant canal" in preference to such special terms as "radial tube" and "intercanal" used by Haeckel for the *Calcarea*. The term "gastral cavity" I pro-



pose to retain in the present paper, because, although the gastral cavity corresponds functionally to the oscular tube of siliceous and horny sponges, it is very improbable that the two structures are homologous.

### The Inhalant Pores.

According to Haeckel (7), in certain of the Sycons a portion of the inflowing water obtains direct access to the tubular flagellated chambers by means of "dermal ostien" situate at the distal extremity of the latter. This never takes place in *Grantia labyrinthica*, although Carter maintains, as I have already mentioned, that the pore-sieves are placed over, and lead into the distal ends of the chambers. As a matter of fact, the pore-sieves, or pore-areas, lie between the ends of the flagellated chambers, and over the ends of the inhalant canals. The blind ends of the flagellated chambers, on the other hand, are covered over by the well-developed dermal cortex.

Each group of pores—for which we may conveniently use the term pore-area as in other sponges—is usually more or less oval in outline, and contains a dozen or more small round pores (fig. 30). The longer diameter of the pore-areas averages about 0.33 mm. in length, and the diameter of the pores themselves about 0.033 mm. The pore-areas and pores are best studied in tangential sections of the dermal surface, a method the importance of which cannot be too strongly insisted upon. In the pore-areas the cortex is reduced to a mere thin membrane, corresponding to the dermal membrane of other sponges (e. g. *Monaxonider*), containing large numbers of small oxeote spicules, and perforated by the pores. If the section be very thin—only of about the thickness of the dermal membrane—it is not easy to determine the boundaries of the pore-areas, which lie very close together. If, however, the section be fairly thick, then a portion of the mesodermal trabeculae separating the subdermal cavities will be included, and the appearance shown in fig. 30 will be presented, where each pore-area is seen overlying the end of an inhalant canal. Fig. 31 shows a single pore more highly magnified.

### The Inhalant Canals.

These commence as widely expanded cavities immediately underlying the pore-areas. These cavities correspond in position to the subdermal cavities of other sponges, but they merge so gradually into the deeper parts of the inhalant canals, with which they are directly continuous, that it is impossible to distinguish the boundaries between the two. The inhalant canals (intercanals of Haeckel) are by no means regular; they may anastomose and they may branch. The anastomosis takes place—most frequently, at any rate—just below the surface, so that the pores of two contiguous areas may lead almost directly into one and the same inhalant canal. The branching takes place chiefly at the far end of the canals, towards the gastral surface.

At first very wide, the inhalant canals, as they penetrate below the dermal cortex and between the flagellated chambers, rapidly diminish in diameter, and finally come to an end just below the gastral cortex where the flagellated chambers are just commencing (figs. 25, 26, *in. c.*). In figs. 28 and 29, which represent sections taken at right angles to the long axes of the flagellated chambers, the inhalant canals are seen cut transversely between the chambers. In fig. 28, which represents a section taken not very far from the middle of the sponge-wall, the inhalant canals are still wide; but in fig. 29, which represents a section from near the gastral surface, the inhalant canals have become very much reduced in size.

### The Prosopyles.

The term "prosopyle" is used by Sollas (9) to designate the openings of the inhalant canals into the flagellated chambers, and to distinguish them from the inhalant pores on the surface of the sponge. As it is a decided advantage to employ two separate terms for these two very distinct structures, I shall adopt Sollas's nomenclature.

The prosopyles in *Grantia labyrinthica* are numerous small circular apertures, each about 0.018 mm. in diameter,

which place the inhalant canals in direct communication with the flagellated chambers. On looking down upon the wall of a chamber the prosopyles are seen fairly regularly scattered over it (figs. 23, 25). In this way they are most readily recognised, but in order to see the actual communication between the flagellated chambers and the inhalant canals it is necessary to examine very thin sections. We then see that the amount of mesodermal tissue intervening between the inhalant canals and the chambers which they supply is by no means great. On approaching a prosopyle (fig. 26, *pr.*) it thins away altogether, and the flattened epithelial lining of the inhalant canal meets the lining of collared cells of the chamber around the margin of the circular aperture. The prosopyles are scattered over the whole of the chamber, so that some are found quite close to the exhalant aperture (fig. 26); in such cases the water can only just enter the chamber and leave it again almost immediately.

Mr. Carter gives a very different account of the prosopyles in *Grantia labyrinthica*, which I have already quoted on a previous page. They do not, as he suggests, "serve for the purpose of intercommunication where the walls of the neighbouring chambers are in direct contact with each other, or for the purpose of allowing the ova developed in the intercameral tissue to pass into the chamber and thus be expelled." In the first place it appears, as I shall show presently, that the walls of neighbouring chambers never are in direct contact with each other, nor have I found their cavities ever in direct communication; and, secondly, I have been able to prove (10) that the embryos escape in a very different manner from that suggested.<sup>1</sup> In short, the prosopyles of *Grantia labyrinthica* agree in function and position with those of other Sycons as described and figured by Schulze (11), Poléjaeff (8), and others.

#### The Flagellated Chambers.

These have the usual Sycon character of more or less

<sup>1</sup> Cf. next page.



cylindrical tubes penetrating the sponge-wall at right angles to its two surfaces—not extending, however, completely from surface to surface, but terminating at either end just beneath the cortex (figs. 23, 25—27). In transverse section (figs. 28, 29) the chambers appear approximately circular, or at all events more or less rounded in outline, and not, as in many Sycons, polygonal from mutual pressure. The retention of the primitive cylindrical character is doubtless due to the fact that the chambers are not very closely packed, but separated by a fair amount of intervening mesoderm. At their peripheral ends the chambers terminate blindly beneath the dermal cortex, there being, as already stated, no dermal ostia to place them in direct communication with the exterior. At their peripheral ends also the chambers exhibit a marked inclination towards branching. I have endeavoured to represent the most striking instance of this which has come under my notice in fig. 23. This tendency towards branching of the chambers appears to be not very uncommon amongst the Sycons, and is mentioned by Schulze in the case of *Sycandra raphanus* (11). I hope to be able to discuss its possible significance at a later date.

In specimens, or in those parts of specimens which contain pretty far advanced embryos, the walls of the flagellated chambers are frequently seen to exhibit little shallow pits on their inner surface (fig. 23, *em. c.*). These little pits or pockets, instead of being lined by the usual collared cells, are lined by flattened pavement-cells. They are the remains of cavities in the mesoderm from which embryos have escaped by bursting through the wall of the chamber and tearing away part of it with them. The collared cells of the part torn away first become stretched out and flattened, as shown in fig. 38, by the pressure of the growing embryo beneath them; finally they appear to degenerate altogether, so as to form a structureless membrane, which is carried away bodily by the escaping embryo. For further particulars as to the mode of escape of the embryos the student is referred to my paper "On the Pseudogastrula Stage in the Development of Calcareous Sponges" already cited.

The stream of water leaves the flagellated chamber through the gastric ostium or exhalant aperture, a wide opening guarded by a delicate, membranous, sphincter diaphragm, as already described (4) by Carter (vide figs. 23—26).

In sections such as that represented in fig. 29 I have not infrequently met with peculiar structures having the appearance shown at *x* in the figure. These structures evidently represent some probably normal phase in the life-history of the flagellated chambers, and it appears to me not improbable that they may be chambers in process of dying.

That the Sycon chamber, as an individual, can die, is perhaps a somewhat novel idea; but if, as everyone will admit, an Ascon individual dies, there is no difficulty in supposing that a single chamber of a Sycon, which in many respects corresponds to an entire Ascon, should also die. Indeed, it is evident from the ontogeny of *Grantia labyrinthica* (vide infra) that as the stalk develops the first formed chambers must perish (fig. 27); and if so, why not individual chambers later on? Haeckel, in his work on the 'Challenger Deep-sea Keratosa' (12), suggests that the flagellated chamber is to be regarded as the individual, comparable to an individual person of a hydroid colony; and in accordance with this view we may certainly expect to find individual chambers perishing, while the sponge as a whole continues to exist healthily.

In the cases alluded to (fig. 29, *x*), the skeleton of what I believe to have been an originally normal and healthy chamber is still present in exactly its normal position and enclosing the normal space. This space, however, is no longer completely filled by the chamber, but the latter has shrunk away from the surrounding tubar skeleton into the centre, where it occupies little more than a third of its original diameter. The remains of the collared cells are distinctly visible, closely, if not exactly, resembling the collared cells which line the surrounding chambers, only less regularly arranged and in more than one layer. In the process of shrinking the meso-

derm around the chamber has been pulled out into delicate strands forming a kind of network, but mostly radially disposed, and thus serving to suspend the dying or dead chamber as shown in the figure.

Fig. 28 (*x*) shows what is perhaps a later stage in events. The chamber occupies a still smaller space, and the surrounding mesoderm has become solid and homogeneous again. The arrangement of the spicules and of the surrounding chambers still indicates the space originally occupied by the dying chamber.

If the individual chambers die it is probable that they are replaced by new chambers ; and, indeed, I shall give reasons later on, in describing the exhalant canals, for supposing that new chambers are actually interpolated between the old ones. Thus the older parts of the sponge may be kept alive and vigorous by the gradual replacement of the old flagellated chambers, as they reach their limits of existence and die off, by new ones. I have unfortunately found no evidence to show how the new chambers originate, but since the older flagellated chambers frequently branch it is not unlikely that they may also bud, or the new chambers may be developed from amœboid cells as in the embryo of *Stelospongos* (2). The problem is on much the same footing as the question, how are new chambers constantly added around the margin of the growing sponge-cup ? and, so far as I am aware, no one knows. All I can say is that they commence life very small, and gradually increase in size as they grow older (figs. 25, 27) ; they make their first appearance in about the middle of the thickness of the sponge-wall, and apparently do not originate as out-growths of the gastral cavity.

Another explanation of the unusual condition of the flagellated chambers described above is suggested by some observations of Sollas, in his report on the "Challenger" Tetractinellida (13), to the effect that the walls of the flagellated chambers in this group sometimes appear contracted, under which condition "fine filaments may be frequently observed produced from the base of the choanocytes and extending



radially from the chambers into the surrounding matrix." "The whole appearance is suggestive of a contraction of the choanocytal wall under the influence of some strong stimulus, possibly of the alcohol into which the sponge was plunged on removal from the dredge."

Doubtless the unusual appearances of the chambers observed by Sollas and myself are in both cases due to contraction; and had I seen only such cases as that represented in fig. 29, *x*, I should have been strongly inclined to regard the contraction either as a merely temporary condition or as a post-mortem condition, produced, as suggested by Sollas, by the action of the alcohol. The appearances presented in fig. 28, however, in which the gelatinous matrix around the chamber has regained its normal condition, while the chamber is more contracted than ever, seem to me to demand a different explanation, which I have endeavoured to give above.

#### The Exhalant Canals.

Since the flagellated chambers do not extend to the actual gastral surface, but are separated therefrom by the entire thickness of the gastral cortex (fig. 26), the existence of special exhalant canals becomes necessary in order to place the chambers in communication with the gastral cavity. These exhalant canals are short, wide, cylindrical tubes, sharply marked off by the sphincter diaphragms already described from the chambers at the one end, and opening directly, without any narrowing or diaphragm, into the gastral cavity at the other (fig. 26).

I have already had occasion to mention that sometimes an unusually small exhalant canal is met with, cut transversely, in tangential sections of the cup wall a little below the level of the ordinary exhalant canals. Such small canals are surrounded by quadriradiate spicules of a slightly unusual form (fig. 21). I am at a loss to explain the existence of these smaller canals, with their slightly peculiar spicules, unless they be simply the exhalant canals of young interpolated flagellated chambers, surrounded by young spicules. This view is supported by the

fact that the spicules in question are somewhat smaller than the more ordinary quadriradiates.

### The Gastral Cavity and Osculum.

As already pointed out in my description of the external form of the sponge, the gastral cavity and osculum are greatly modified by the peculiar mode of growth of the sponge. The gastral cavity, instead of being narrow and tubular, has become wide and basin-like, and at the same time, owing to the convolutions of its wall, extremely irregular. The osculum has thus become wider than any other part of the gastral cavity—a condition the opposite of that which obtains in other Sycon sponges.

Sometimes traces of the gastral cavity may be found in the stalk even of adult sponges, causing the latter to be more or less hollow. This indicates that the gastral cavity originally extended all through the sponge—a fact which is proved, as I shall show later on, by the ontogeny.

#### (e) The histology of the soft tissues.

The terms ectosome and choanosome, proposed by Sollas (15) and adopted by myself (2) in describing siliceous and horny sponges, are not convenient for at any rate the great majority of the Calcarea, and it is better to classify the tissues simply under the heads ectoderm, mesoderm, and endoderm. I must follow the example of Schulze (14) in considering that the ectoderm of the larval sponge (in the case of the Sycons, at any rate) furnishes not only the epithelium of the dermal surface, but also the epithelial lining of the inhalant canal system; while the endoderm lines the remainder of the canal system from the prosopyles to the margin of the osculum, and the mesoderm furnishes all the remainder of the sponge body.

### The Ectoderm.

The ectoderm resembles exactly what Schulze has described (11) in *Sycandra raphanus*, consisting of a single layer of flat, polygonal epithelial cells lining the dermal surface of the sponge and the inhalant canal system. These cells are most

readily distinguished around the inhalant canals, where they are less obscured by spicules and other mesodermal structures than on the dermal surface. The nucleus is surrounded by the very characteristic granules described by Schulze in *Sycandra*. In my preparations I have only after some trouble succeeded in making out the boundary lines between the individual cells, and Schulze himself observes that it is remarkable that the boundaries of these cells—sometimes so distinct—are not always clearly visible. Nevertheless I have been able to determine the shape of the cells pretty accurately, and found them to agree precisely with Schulze's drawings.

#### The Endoderm.

This consists, as in other Heterocæla, of two parts: (1) a layer of flattened epithelial cells lining the gastral cavity and the short exhalant canals of the chambers; (2) a layer of collared cells lining the flagellated chambers themselves. The epithelial portion of the endoderm exhibits no features of special interest and needs no further description, so we may pass on at once to the collared cells.

Dr. R. von Lendenfeld has recently (16) called in question the accuracy of my description of the collared cells, with their connecting membrane, in *Stelospongos* (2), observing, "Es ist jedoch seine schematische Darstellung dieser Membran (Taf. xxxii, fig. 9) keineswegs Vertrauen-einflössend, sondern eher ein Beweis der theoretischen Unwahrscheinlichkeit der Existenz derselben." Notwithstanding this criticism, I still maintain the correctness of my original description and figures, and have already published a note (17) in the 'Zoologischer Anzeiger' in reply to Dr. von Lendenfeld's observations. The latter are apparently based partly on imperfect observation, and partly on the convenient, albeit somewhat unphilosophical, assumption that all sponges must be exactly alike in this respect. Dr. von Lendenfeld finds that in certain sponges examined by him "der Raum zwischen den Kragenzellen von einer durchsichtigen, der gewöhnlichen Grundsubstanz der Zwischenschicht der Spongien sehr ähnlichen Substanz



ausgefällt sei;" and the only conclusion at which he is able to arrive with regard to the question of Sollas's membrane is that it does not exist, and that Professor Sollas and I have only misinterpreted what it has been reserved for him to correctly describe. But in spite even of a preconceived "theoretische Unwahrscheinlichkeit" I adhere to my original opinion. For my own part, I am unable to see where the theoretical improbability comes in. Quite recently Mr. Carter has, in a private letter, afforded me valuable corroborative evidence of the existence of Sollas's membrane. He says, "I have seen in the brim of the collar of the Calcsponge spongozoon plastic amalgamation like that produced by two semi-fluid bits of gum—under which circumstances, if all became amalgamated, then you would have Sollas's membrane. Might it not so happen that at one time they may be so amalgamated and at another not, and thus produce the difference?" That Sollas's membrane originated in the almost accidental manner here indicated there can be no doubt, but I am inclined to think that in many sponges it has become a more or less fixed and constant character—a view supported by the fact that, as I shall show later on, it is still recognisable when both collars and flagella are withdrawn. It is exceedingly likely from the nature of the case that it may have originated independently in several groups, so that in each group forms with and forms without it may exist. If so it is only another instance of that homoplasy so characteristic of the Porifera.

I am not aware that there is anything particularly new in Dr. von Lendenfeld's observation that "die Kragenzellen stehen nicht frei auf der Oberfläche der Zwischenschicht, sondern sie sind in dieselbe Eingesenkt" (16). Indeed, in my paper on *Stelospongos* (2) I have said, "I have not been able to trace any definite outline to the body of the cell which is embedded in the highly granular ground-substance." It is amusing to see Dr. von Lendenfeld so vigorously opposing one of my observations which does not happen to fit in with his idea of the fitness of things, and at the same time taking

another from my very next page and putting it forward as though it were original.

Sollas's membrane, however, and the intercellular substance which exists between the bases (and, so far as I have seen, between the bases only) of the collared cells have nothing whatever to do with one another. The former is endodermal in origin, and is separated by a wide empty space from the probably mesodermal ground-substance between the collared cells. In the case of *Stelospongos*, if the mesodermal ground-substance really filled up the whole of the intervals between the collared cells it would be at once recognisable by its highly granular appearance; but it does not, as a glance at my figures will show; it stops at the bottom of the neck.

I must now describe the condition of things in *Grantia labyrinthica*. All the collars and flagella of the collared cells are retracted in my preparations. This is not to be regarded as a purely artificial and post-mortem condition, but probably rather as a periodically recurring phase in the life-history of the cells. Carter has shown long since (18) that the individual collared cells may become amœboid, and probably in the living sponge they often spontaneously retract their collars and flagella and enjoy a period of rest.

In this retracted condition the collared cells (figs. 32, 33) of *Grantia labyrinthica* are somewhat pyramidal bodies, polygonal in transverse section, and with the narrow end pointing towards the lumen of the chamber. They measure about 0·0048 mm. in height and about the same in breadth at the base. The nucleus is situate in the apex of the pyramid (fig. 33). This position of the nucleus appears at first sight a little curious, but it is interesting to observe that it agrees with the position of the nucleus in the long prismatic cells of the embryo (fig. 38) from which the collared cells of the adult are admittedly derived. Thus the collared cells in a state of rest revert more or less to their embryonic condition, the chief distinction being that they are now very much shorter.

Even at their bases the collared cells appear to be separated from one another by distinct intervals (fig. 32), but these may

possibly be due to shrinkage. The apices of the cells are still further apart, and in longitudinal sections (fig. 33) are seen to be connected by a fine, sharp line running from one to the other. This line is Sollas's membrane seen in section, no longer supported on the tops of the collars, which have been retracted, drawing the membrane after them close down into the apices of the cells. So Sollas's membrane remains visible even when the collars of the cells are retracted, which indicates that it is probably a more or less permanent structure, and no mere temporary fusion of the margins of adjacent collars.

Owing to the great transparency of the gelatinous mesodermal ground-substance—which is a very characteristic feature of calcareous sponges—it is not possible to determine, as in the case of *Stelospongos*, exactly how far it extends between the collared cells. Probably there is considerable individual variation in this respect, and it is a matter of little importance to ascertain exactly how far each cell happens to be sunk in the matrix. I cannot believe, however, that the actual collars are ever embedded.

I understand that Mr. Bidder has already pointed out that Sollas's membrane occurs in a calcareous sponge, *Leuconia aspera*, but I have neither been able to see his paper nor to find out where it was published.

### The Mesoderm.

The constituents of the mesoderm may be divided into two main classes, the cells and the cell-products. We will consider the cell-products first; they consist of the intercellular ground-substance and the spicules, and as the spicules have been already fully described we have only to deal with the ground-substance. The ground-substance<sup>1</sup> consists of the usual transparent jelly, exhibiting no differentiation excepting a slight concentration around the spicules, forming the so-called spicule sheaths. These are very distinct, and are always seen to be continuous with the surrounding ground-substance when the spicules themselves have been dissolved out by the

<sup>1</sup> Maltha of Haeckel (12).



action of weak hydrochloric acid. This view regarding the nature of the spicule sheaths has been already expressed by Haeckel and Schulze (11), and is doubtless the correct one.

The mesodermal cells, which lie embedded in the ground-substance, may be classified as follows :

- (1) Amœboid.
- (2) Stellate.
- (3) Glandular :
  - (a) Spicule-secreting.
  - (b) Slime- or cuticle-secreting.
- (4) Endothelial.
- (5) Muscular.
- (6) Nervous.
- (7) Reproductive.

**Amœboid Cells.**—Concerning the amœboid cells proper I have no information to add to that which we already possess. They are distinguished from the stellate cells by their more rounded and massive form, and their more abundant and more granular protoplasm. Certain of them, as is well known, develop into ova, and these I shall describe later on.

**Stellate Cells.**—These, which may be regarded as the connective-tissue cells of the sponge body, have also the usual form, characterised by the long, slender, and often branched processes given off from an inconspicuous central mass of protoplasm surrounding the nucleus (fig. 26, *st. c.*). It is very probable that, as in the case of *Stelospongos* (2) and other sponges, adjacent stellate cells may be united by their slender processes, but I have not succeeded in clearly demonstrating the connection here.

**Glandular Cells.**—These are of two kinds—spicule-secreting, and slime- or cuticle-secreting cells. We will consider the spicule-secreting cells, or calcoblasts, as they have been termed by Poléjaeff (8), first.

It is generally admitted that both calcareous and siliceous spicules originate within special mother-cells, but probably in both cases they subsequently receive additional layers of the calcareous or siliceous material from other cells. Poléjaeff (8)

figures a "conjectural calcoblast" attached to the outside of a spicule of *Leuconia multiformis*. This cell has the form of an ordinary stellate mesodermal cell, and such, so far as structure is concerned, I believe the calcoblast to be. In the horny sponges the corresponding spongioblasts, although somewhat specialized in form, are clearly only slight modifications of stellate cells, as I have elsewhere shown (2). In the calcareous sponges the calcoblasts have acquired the function of secreting carbonate of lime without undergoing any corresponding modification in form. It should, however, be borne in mind that there are probably two kinds of calcoblasts, primary and secondary. The primary calcoblasts are the mother-cells in which the spicules originate, and the secondary calcoblasts are the cells which secrete additional layers of calcareous matter around the spicule after it has been formed. In the same way I have no doubt that there are primary and secondary silicoblasts.<sup>1</sup>

The slime- or cuticle-secreting cells have, so far as I am aware, not hitherto been observed in calcareous sponges, although well known in the *Keratosa* through the researches of von Lendenfeld. In *Grantia labyrinthica* the gland-cells in question occur in a single layer just beneath the epithelium of the surface of the sponge. They are very distinct and plentiful on the gastral surface (fig. 26, *gl. c.*), but less so on the dermal. Each gland-cell consists of an irregular, granular, nucleated body, which closely resembles an ordinary amœboid cell, but which may be seen under favorable conditions to be connected with the overlying epithelium by one or more processes. Fig. 26 shows the layer of gland-cells beneath the gastral epithelium, and fig. 34 is a more highly magnified drawing of an individual gland-cell and its surroundings, as it appeared under a Zeiss F objective and ocular 4. At the particular spot figured an irregularity in the surface caused the epithelium to be cut somewhat tangentially, while the gland-cell itself was cut vertically; the connection between the two is, however, very well shown. Four processes are shown in this

<sup>1</sup> For an illustration of a primary silicoblast the student is referred to the 'Report on the "Challenger" Monaxonida,' pl. xxi, fig. 13.

instance connecting the gland-cell with the epithelium, but it is very unusual to see so many. Just on the right of the gland-cell the epithelium is raised up by a projecting spicule which does not itself appear in the section. On the dermal surface of the sponge the gland-cells are more like ordinary amœboid cells immediately underlying the epidermis.

The essential agreement of these cells with those described by von Lendenfeld in, for example, *Dendrilla cavernosa* (19) is obvious, both as regards structure and arrangement. According to this author the cells secrete a cuticle which is not to be distinguished from the spongin of the horny fibres. I agree that they probably secrete a cuticle, but, as Vosmaer points out (20), no grounds are given for supposing that this is identical with the spongin, and the fact that similar gland-cells occur in the Calcisponges argues against the assumption. Very possibly the cuticle, after a time, more or less entirely replaces the original epithelium, and not only, as suggested by von Lendenfeld,<sup>1</sup> so much of it as may have been accidentally damaged. Hence, perhaps, arises the difficulty of making out the structure of the surface epithelium.

**Endothelial Cells.**—The embryos, which I have described elsewhere (10), lie each in a separate cavity in the mesoderm around the flagellated chambers. These embryo-containing cavities (fig. 38, *em. c.*) are lined each by a single layer of pavement-cells, which are not to be distinguished in my sections from the pavement-cells already described. These cells I regard with Schulze (11) as being of mesodermal origin, and hence endothelial.

In my memoir on *Stelospongos flabelliformis* (2) I regarded the embryo-containing cavities in that sponge as probably, though by no means certainly, specialized parts of the exhalant canal system. As the result of further study, however, I must relinquish this view, and regard the cavities as special excavations in the mesoderm, and the remarkable giant pavement-cells which line them as of mesodermal origin.

<sup>1</sup> For an abstract of von Lendenfeld's observations on the subject vide 20.



**Muscular Cells.**—Certain spindle-shaped cells lying in the membranous diaphragms of the exhalant apertures of the flagellated chambers are probably muscular in function. The cells in question are shown in fig. 24, which renders further description unnecessary.

**Nerve-cells.**—The only cells which I have found in *Grantia labyrinthica* to which a nervous function can possibly be assigned are certain structures which occur around the margins of the inhalant pores, as shown in fig. 31. These cells are elongated radially in relation to the circular pores which they surround. The nucleus is distinct and is placed at the distal end of the cell, and the main mass of protoplasm stretches from the nucleus to the edge of the pore, where it ends in an expansion along the free margin. There may also be indications of smaller processes given off near the base of the cell. It is probable that the thickening of the main protoplasmic process of each cell as it touches the margin of the pore may simply indicate a retracted, sensitive, hair-like process such as Stewart (21) and Lendenfeld (22) describe; but it seems also just possible that each nerve-cell naturally ends in a sensitive plate or expansion at the free margin of the pore. If we adopt the former of these two views the sensitive cells will be seen to agree pretty closely in structure with those described and figured by Stewart in *Grantia compressa*; but I have met with no evidence of the grouping of the cells into "synocils," as described and figured by von Lendenfeld. Von Lendenfeld, however, has also described single sensitive cells ("Sinnes-Ganglienzelle") around the inhalant pores of his *Chalinissa communis*, var. *flabellum* (23), which in structure and arrangement almost exactly agree with those found by me in *Grantia labyrinthica* except that he figures the end of the main protoplasmic process projecting for a short distance beyond the margin of the pore. This slight difference may, as will be gathered from what I have already said, be due to differences in state of contraction in the two cases. It is interesting to note that the sense-cells of *Grantia labyrinthica* agree more closely with those of so different a sponge as

*Chalinissa communis* than they do with those of the closely related *Grantia compressa*. We must associate with this fact the fact that the arrangement of the pores in *Grantia labyrinthica* also agrees much more closely with that which usually obtains in the *Chalininæ* than with that found in *Grantia compressa*, thus affording another instance of homoplasy.

The cells which Sollas (13) describes and figures as possible sense-cells ("æsthocytes") in the Tetractinellida appear to be of a more problematical character.

**Reproductive Cells.**—The only reproductive cells which I have met with are the ova. These, as in other sponges, are obviously derived from amœboid cells, and in the earliest stages of their development it is impossible to distinguish between the two. Later, however, the ova become more rounded off, and the nucleus becomes large and distinct.

It is now generally admitted that the ova of sponges are fertilised by spermatozoa probably of other sponges, which gain admission to the sponge with the inflowing current of water. No one, however, so far as I can discover, has attempted to find out whereabouts in the sponge the union of ovum and spermatozoon takes place. Having followed the spermatozoon into the canal system they leave it there to take care of itself, forgetting that, unless some special arrangement exists to prevent such a catastrophe, it will speedily be washed out again through the osculum without ever having had a chance of fulfilling its errand. The general assumption would seem to be that the spermatozoon loses its way through the walls of the canals, and wanders about in the gelatinous mesoderm until it happens to come across an ovum. It seems highly improbable that this should be the case, for it would be a strange thing if the spermatozoa bored their way through the epithelium, as they would have to do in order to get into the gelatinous ground-substance, without any obvious inducement to do so. Mr. Carter seems to have come nearer to the truth than anyone, but without realising the true significance of what he saw. He says (24) that the ovum in *Grantia com-*

pressa "may be seen to be hanging, pear-shaped, upon the surface of the excretory canals, where it remains for a certain time locomotive, until, after further development, it becomes permanently fixed, and the locomotive envelope seems to pass into a capsule." It is obvious that by "excretory canals" Mr. Carter means the inhalant canals, and he is probably wrong in considering that the locomotive envelope of the ovum passes into a capsule; but the main fact, that the ovum at a certain stage of its existence hangs freely from the surface of a portion of the canal system, is clearly brought out.

In *Grantia labyrinthica* I have distinctly observed the ova hanging from the epithelial lining of the inhalant canals by means of short peduncles, and projecting freely into the lumen of the canal, where they must be washed by the incoming stream of water. Fig. 35 represents an amœboid ovum approaching the surface of an inhalant canal; and figs. 36 and 37 represent it, after having passed through the epithelium, hanging freely by its short peduncle, awaiting fertilisation. In fig. 37 the nucleus of the ovum appears very near the surface, in a position suggestive of the formation of a polar body. Probably the ova pass through the epithelium of the inhalant canals in the same way that the white blood-corpuscles pierce the walls of the capillaries in higher animals.

After fertilisation the ovum probably migrates back into the gelatinous ground-substance, and takes up its position near the wall of a flagellated chamber, there to undergo the earlier stages of its development. This seems more probable, from what we know (10) of the position of the embryos, than the supposition that it remains and develops in the spot where it is fertilised.

It is probably a general rule in sponges that the ova are fertilised while hanging from the walls of the canal system, and that they migrate first of all through the canal-wall to be fertilised, and then back again into the gelatinous ground-substance to undergo development; hence the necessity for the amœboid movements so characteristic of sponge ova. Thus we see that the sponge ovum plays an unusually active part in the process of fertilisation, as it were meeting the spermatozoon



halfway. The migrations of the ova in sponges remind one forcibly of Weissmann's descriptions of the migrations of the ova in various hydroids (25).

### The Post-embryonic Development of *Grantia labyrinthica*.

The embryology of *Grantia labyrinthica*, so far as the material at my disposal has allowed me to work it out, forms the subject of a special memoir (10), so that it is unnecessary for me to deal with the question in this place. The post-embryonic development, however, has not as yet been dealt with, and although there is but little to be said about it, yet that little is of interest as clearly indicating the manner in which the very peculiar external form has been evolved.

Fig. 1 shows the youngest stage found, in which it will be seen that, as regards both size and shape, the sponge differs but little from an ordinary *Grantia*. The wall of the tube is even and not convoluted, although already the osculum is the widest part of the gastral cavity. Fig. 27 represents somewhat diagrammatically a longitudinal section of this specimen, and is important chiefly because it shows how the stalk arises by the filling up of the lower portion of the gastral cavity with a copious growth of mesodermal tissue in which numerous spicules are developed. As I have already pointed out, remnants of the gastral cavity may sometimes be recognised in the stem even of adult specimens. Figs. 2 and 3 represent two stages intermediate between that just described and the full-grown sponge (fig. 4). The specimen drawn in fig. 3 is very much compressed, so as to be bilaterally symmetrical. This may either be accidental, or it may indicate the commencement of folding in the wall of the cup. All figures are drawn of the natural size, and they render further description needless.

I may conveniently here describe the only case of budding with which I have met in the species. On the outer surface of the wall of the cup in a well-grown specimen, and not far below the margin, a small individual had budded off

from the parent sponge. This is represented, twice the natural size, in fig. 5. The gastral cavity of the young sponge is still connected with that of the mother by a very evident aperture, and it is interesting to notice that in this case the osculum is distinctly constricted, so that the bud has the typical Sycon form.

### The So-called Family Teichonidæ.

The very artificial group of sponges now generally known under the name Teichonidæ has been unfortunate from first to last. It was not even christened properly, for the title of Mr. Carter's original paper (3) is printed "On Teichonia, a New Family of Calcareous Sponges, with Descriptions of Two Species;" while subsequently, on the same page, the name Teichonellidæ is given to the new family, which is concisely diagnosed as "vallate." Then the genus Teichonella is diagnosed as follows: "Vallate or foliate, without cloaca. Vents numerous, confined to the margin or general on one side of the lamina only; naked."

Teichonella prolifera is described as the first species of the genus, and in the same paper Grantia labyrinthica is described as a second species. Subsequently, as I have already said, Mr. Carter withdrew Grantia labyrinthica from the genus Teichonella, and recognised its true position amongst the Sycons.

Now we see upon what slight foundations spongologists build their edifices. Poléjaeff (8) adopts the new family, altering the name, however, to Teichonidæ. He states that the main character of the family consists in the differentiation of the outer surface into two planes, one bearing oscula, the other pores exclusively; and he enters into a curious speculation as to whether a Teichonid is "a colony with dislocated oscula and pores," whatever that may be. But what authority had Poléjaeff for stating that the outer surface was differentiated into two planes, one bearing oscula and the other pores? Mr. Carter never said so. His diagnosis of the family is "vallate," while even his generic diagnosis says nothing about

the pores. In the description of the type species (*Teichonella prolifera*), however, we find the words "pores invisible to the naked eye, scattered over the surface thickly, and generally vents slightly margined, . . . arranged more or less in a single line along the margin only." I have myself most carefully examined *Teichonella prolifera* by means of stained sections cut by the paraffin method, and I find that it is nothing but an ordinary *Leucon*, with pores on both surfaces of the low, thick walls of which the sponge consists, and oscula along the raised margin (fig. 6). The surface is certainly not differentiated into anything like a pore-bearing and an osculum-bearing plane.

The fact appears to be that Poléjaeff wanted a place for his new genus *Eilhardia*, and so he seized upon the *Teichonellidæ* and altered both the name and the characters of the family to suit his own ideas, apparently without having so much as ever seen a specimen of *Teichonella*. Of course, both *Teichonella* and *Eilhardia* duly appear in an elaborate genealogical tree.

*Eilhardia*, if Poléjaeff's figures be correct, is a *Leucon*. True, the pores appear to be on one surface and the oscula on the other, but can anyone possessed of the slightest knowledge of the subject regard this as a family character? I think certainly not, and in support of my opinion venture to call attention to the following extract from Ridley and Dendy's report on the "Challenger" Monaxonida (26):—"Before leaving the question of the pores we must consider briefly the condition of flabellate sponges in this respect. It is an almost invariable rule that in flabellate sponges the pores are to be found on one surface and the oscula on the other. Thus in *Phakellia ventilabrum*, var. *connexiva* (pl. xxxv, figs. 3, 3*a*; pl. xlix, fig. 3), and *Phakellia flabellata*, nobis (pl. xxxiv, figs. 2, 3, 3*a*), this arrangement is very well illustrated; and the same condition occurs in *Myxilla frondosa*, nobis (pl. xxvi, figs. 1, 1*a*), and *Gellius flabelliformis*, nobis (pl. xxvi, figs. 5, 5*a*). Again, in that very remarkable sponge, *Esperiopsis Challengeri* (pl. xviii), the pores

occur only on the concave surfaces of the lamellæ (pl. xviii, fig. 4), while the oscula are all on the convex surfaces.

“By far the most remarkable instance of this kind is, however, afforded by a boring Suberite which we have described under the name *Cliona dissimilis* (p. 227, pl. xxv, fig. 5, &c.). Here the sponge has bored its way into a flattened coral which it completely surrounds; hence it has itself acquired a flattened, lamellar form, and we find the pores collected in areas (woodcut, fig. 11, *pa.*) on one side of the sponge, and the oscula (woodcut, fig. 11, *o.*) on the other.”

“There is no other known example, so far as we are aware, of a lamellar Suberitid sponge; and even the species in question is lamellar only because it has bored into a lamellar coral, and yet the pores and oscula are arranged just as they would be in a free-living frondose sponge, such as *Phakellia*. There must be some strong reason why as soon as a sponge, for any cause, acquires a lamellar form, the oscula become confined to one surface and the pores to the other, and to account for the occurrence of this condition in genera so widely separated as *Gellius*, *Myxilla*, *Phakellia*, and *Cliona*. What this reason may be we cannot at present say.”

I am still no better able to give an explanation of this curious phenomenon than I was when the above passage was written; but the facts appear to me to be conclusive evidence against the value of the peculiar arrangement of the pores and oscula as a family character.

But even if it were allowed that the arrangement of the pores and oscula were a character of family importance we could not put *Eilhardia* and *Teichonella* in the same family, for, as I have shown, they differ widely from one another in this respect. Then, according to Poléjaeff's diagnosis, not *Eilhardia*, but *Teichonella*, would have to come out of the family *Teichonidæ*. As a matter of fact the family ought to be abandoned altogether, and the three species which have been at various times placed in it distributed as follows:



|                              |   |   |   |   |                   |
|------------------------------|---|---|---|---|-------------------|
| <i>Teichonella prolifera</i> | . | . | . | . | <i>Leuconidæ.</i> |
| <i>Eilhardia Schulzei</i>    | . | . | . | . | <i>Leuconidæ.</i> |
| <i>Grantia labyrinthica</i>  | : | . | . | . | <i>Syconidæ.</i>  |

It would not have been necessary to deal with this question so carefully had not Poléjaeff's emended family *Teichonidæ* met with such general and unquestioning acceptance. Thus Vosmaer adopts it in his most important work (20), and Lendenfeld (6) gives it a place in his system and in the inevitable genealogical tree. Haeckel also accepts the family in his latest work on sponges (12).

In conclusion I wish again to record my great indebtedness to Professor Howes for his kindness in correcting the proofs of this paper in my absence from England.

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## DESCRIPTION OF PLATES I—IV,

Illustrating Mr. Arthur Dendy's paper "Studies on the Comparative Anatomy of Sponges. III.—"On the Anatomy of *Grantia labyrinthica*, Carter, and the So-called Family Teichonidæ."

## PLATE I.

FIGS. 1, 2, 3.—Three early stages in the post-embryonic development of *Grantia labyrinthica*. Nat. size.

FIG. 4.—Adult specimen of *Grantia labyrinthica*, growing upon a mass of foreign matter. Nat. size.

FIG. 5.—Portion of an adult specimen of *Grantia labyrinthica*, with a bud attached to the outer surface.  $\times 2$ .

FIG. 6.—Specimen of *Teichonella prolifera*, showing the oscula distributed along the raised margin. Nat. size.

## PLATE II.

SKELETON OF *GRANTIA LABYRINTHICA*.

FIGS. 7, 8, 9.—Large linear spicules (oxea) from the margin of the osculum. Zeiss, D, ocular 2, camera.

FIGS. 10, 11.—Oxea from the surface of the cup. Zeiss, D, ocular 2, camera.

FIG. 12.—Large oxeote spicule from the surface of the stem. Zeiss, D, ocular 2, camera.

FIGS. 13, 14, 15, 16, 17, 18.—Different forms of triradiate spicules from the wall of the cup. Zeiss, D, ocular 2, camera.

FIG. 19.—Sagittal triradiate spicule from the stem. Zeiss, D, ocular 2, camera.

FIG. 20.—Quadriradiate spicule from the gastral cortex. Zeiss, D, ocular 2, camera.

FIG. 21.—Arrangement of quadriradiate spicules around the exhalant canal of a young flagellated chamber.

FIG. 22.—Arrangement of the skeleton as seen in vertical longitudinal section of a chamber from gastral to dermal surface.

## PLATE III.

ANATOMY OF *GRANTIA LABYRINTHICA*.

FIG. 23.—A single flagellated chamber and its exhalant canal, in part laid open by longitudinal section. *ex. c.* Exhalant canal. *di.* Diaphragm. *pr.*

Prosopyle. *fl. c.* Cavity of flagellated chamber. *em.* Embryo. *em. c.* Embryo-containing cavity (the embryo has already escaped in this case). The collared cells are coloured red, and the embryos brown. Zeiss, D, ocular 2.

FIG. 24.—Exhalant opening of a flagellated chamber, with its membranous diaphragm (*di.*). *mus.* Muscle-cells. The spicules are coloured blue, and the collared cells (which lie somewhat below the level of the diaphragm) red. Zeiss, F, ocular 2.

FIG. 25.—Part of a section vertical to the margin and to the two surfaces of the wall of the cup. *em.* Embryo. *o. sp.* Spicules of the oscular fringe. *p.* Inhalant pore. *p. a.* Pore-area. *in. c.* Inhalant canal. *g. s.* Gastral skeleton. *d. s.* Dermal skeleton. *t. s.* Tubar skeleton. Other lettering and colouring as before. Zeiss, A, ocular 3.

FIG. 26.—Gastral portion of a thin section similar to the last, but more highly magnified. *gl. c.* Gland-cells lying beneath the gastral epithelium. *st. c.* Stellate cell. Other lettering and colouring as before. Zeiss, F, ocular 2.

FIG. 27.—Vertical section through the young specimen represented in Fig. 1. *gas. c.* Gastral cavity. *mes.* Growth of mesodermal tissue filled with spicules, to form the stalk. Flagellated chambers red, spicules blue.

#### PLATE IV.

##### ANATOMY OF GRANTIA LABYRINTHICA.

FIG. 28.—Portion of a section taken parallel to the surface of the sponge-wall, and somewhat nearer to the dermal than to the gastral surface. It will be noticed that the inhalant canals are still of large size. *s. sp.* Sections of the shafts of the triradiate spicules of the tubar skeleton. *x.* Peculiarly modified flagellated chamber. Other lettering and colouring as before. Zeiss, D, ocular 2.

FIG. 29.—Portion of a section similar to the last, but taken near the gastral surface, showing the diminution in diameter of the inhalant canals, &c. Lettering and colouring as before. Zeiss, D, ocular 2.

FIG. 30.—Portion of the dermal surface sliced off, showing the pores arranged in pore-areas. Some of the spicules are omitted. Lettering and colouring as before. Zeiss, A, ocular 2.

FIG. 31.—A single pore more highly magnified, showing the nerve-cells (*n. c.*) around its margin. Zeiss, F, ocular 2.

FIG. 32.—A group of collared cells (with retracted collars and flagella), seen from above or below. *n.* Nucleus. Zeiss, F, ocular 3.

FIG. 33.—A row of four collared cells (with retracted collars and flagella), seen from the side. *n.* Nucleus. *s. m.* Sollas's membrane shrunk down upon the apices of the cells. Zeiss, F, ocular 3.

FIG. 34.—A single gland-cell (*gl. c.*) connected by four processes with the



overlying epithelium (*ep.*), which latter is cut almost tangentially owing to the unevenness of the surface. *p. ep.* Projection of the surface epithelium around a protruding spicule, which latter is not itself seen in the section. Spicules blue. Zeiss, F, ocular 4.

FIG. 35.—An amœboid ovum (*ov.*) lying in the gelatinous ground-substance of the mesoderm, near the epithelium (*ep.*) of an inhalant canal. Zeiss, F, ocular 3.

FIG. 36.—An ovum (*ov.*) hanging by a stalk from the epithelium (*ep.*) of an inhalant canal. Zeiss, F, ocular 3.

FIG. 37.—Another example of an ovum (*ov.*) hanging by a stalk from the epithelium (*ep.*) of an inhalant canal. Zeiss, F, ocular 3.

FIG. 38.—An embryo (approaching the pseudogastrula stage) lying within the embryo-containing capsule in the mesoderm, between two flagellated chambers of the mother sponge. *col.* Collared cells of mother sponge. *sp.* Spicule of mother sponge. *end.* Prismatic cells (future endoderm) of the embryo. *mes.* Incipient mesoderm of the embryo. *gr.* Layer of large granular cells of the embryo. Other lettering as before.



Studies on the Comparative Anatomy of  
Sponges.

IV.—On the Flagellated Chambers and Ova of  
*Halichondria panicea*.

By

**Arthur Dendy, M.Sc., F.L.S.,**

Demonstrator and Assistant Lecturer in Biology and Fellow of Queen's  
College, in the University of Melbourne.

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With Plate V.

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THE purpose of the present short communication is, as indicated in the title, to call attention to the minute structure of the flagellated chambers and ova in the common British sponge —*Halichondria panicea*.

The material upon which my observations are based I owe chiefly to Mr. Martin Woodward, who kindly gave me some fine pieces of the sponge in question, collected by himself in the English Channel. Some of the material thus obtained was treated while fresh with osmic acid, to which fact is due the excellent state of preservation. Material not so preserved I find to be practically valueless for the study of the histology of the chambers, which, indeed, can only be made out where the osmic acid has penetrated before the death of the sponge.

Part of the material was dredged by myself in the Solent, and it was in sections of this that I first recognised the existence of Sollas's membrane in the species.<sup>1</sup> The sections were

<sup>1</sup> "Studies on the Comparative Anatomy of Sponges," II, 'Quart. Journ. Micr. Sci.,' December, 1888.

stained with borax carmine, and cut by the ordinary paraffin method.

I at one time hoped to be able to publish a complete account of the minute anatomy of *Halichondria panicea*; but my removal from England and the pressure of other work render this impracticable, and I must content myself with describing only the most important points. This is particularly desirable, because I find in this sponge the best example with which I have yet met of Sollas's membrane, the very existence of which has lately been called in question by Dr. R. von Lendenfeld.<sup>1</sup> I need not here reply to the arguments of this observer, because I have already done so in my article on "Some Old and New Questions concerning Sponges," in the '*Zoologischer Anzeiger*,'<sup>2</sup> and more fully in the third of my "Studies on the Comparative Anatomy of Sponges," recently sent to England for publication in the '*Quarterly Journal of Microscopical Science*.' To these papers and to the second of my "studies," already cited, I must refer the reader for further information, and for the literature of the subject.

*Halichondria panicea* may be so readily obtained in England, that any student of sponges acquainted with the modern method of research can easily satisfy himself as to the correctness of the observations here recorded.

Before describing the flagellated chambers of *Halichondria panicea* it will be advisable to say a few words as to the canal system. This is of the lacunar type. The inhalant pores, scattered over the surface of the sponge, lead into a system of irregular lacunæ, scarcely definite enough to deserve the name of canals. The ectosome is thin, and the subdermal cavities are not recognisable as distinct structures. As they penetrate below the surface, branching again and again, the inhalant lacunæ become smaller and smaller, being recognisable in sections only as minute cavities surrounded by the flagellated chambers. Interdigitating with the inhalant lacunæ in

<sup>1</sup> '*Zoologischer Anzeiger*,' No. 311, 1889, p. 362.

<sup>2</sup> No. 325, 1890.



the most complicated and irregular manner we find the precisely similar exhalant lacunæ. The ultimate inhalant and exhalant lacunæ are separated only by narrow strands of gelatinous mesodermal tissue, in which lie the spicules and the flagellated chambers (fig. 1). Thus every chamber lies wedged in between an inhalant lacuna on the one side, and an exhalant lacuna on the other; and it is only by noticing which way the exhalant openings of the chambers are turned that it is possible to tell whether a given lacuna is inhalant or exhalant. The exhalant lacunæ gradually unite together into larger and larger channels, and open finally on to the surface of the sponge by means of the wide oscula, generally, if not always, passing first into well-defined oscular tubes.

The chambers themselves (fig. 1) may be roughly described as subspherical. When, however, they are cut along the imaginary axis running through the inhalant and exhalant poles they frequently appear somewhat compressed, and we see also that the exhalant opening (fig. 1, *c*, *e. o. c.*) is very wide. When cut in a direction at right angles to this axis they appear circular in outline, and the wall of the chamber is uninterrupted (fig. 1, *a*). The diameter of the chamber in this case is about 0.047 millimetre in length. (In the report of the 'Challenger' Monaxonida the average diameter of the chambers is given as 0.0336 millimetre. This figure appears from my subsequent researches to be rather too low; but it must be remembered that a certain amount of variation occurs in this respect, in accordance with the state of preservation of the specimens examined.)

We now come to the most important consideration, viz. the form and arrangement of the collared cells; and we will first describe them as they appear when the chamber is seen in section (fig. 1, *a, c*). The collared cells stand some little distance apart from one another on the gelatinous ground-substance surrounding the chamber. They have each a short nucleated body, indistinguishable from the collum or neck, and surmounted by the delicate funnel-shaped collar. The outlines of the collars are extremely fine, but all the collars are con-

nected at their margins by a very distinct membrane, which appears in section as a thicker line running from one to the other, but interrupted by the mouths of the collars, as shown in the figure. The structure and relations of this membrane (Sollas's membrane) appear exactly as I have described them in *Stelospongos*. The collared cells nearest to the exhalant opening of the chamber are shorter than those farther away, so that the membrane gradually approaches more and more closely to the gelatinous ground-substance around the chamber, and finally seems to run into it at the opening itself (fig. 1, c). The fact that Sollas's membrane appears here as a thicker line than the outlines of the collars is, I believe, simply due to the thickness of the sections causing us to see a little more than the mere cut edge of the membrane.

Professor Sollas, in his valuable 'Report on the Tetractinellida of the Challenger Expedition,' observes, "I have never yet seen the flagella of the concrescent choanocytes, though I have never failed to find them in the case of choanocytes which are not concrescent. It might be explained on the supposition that the flagella are retracted in the former case; but that naturally leads to the inquiry as to why they are not retracted in the latter." In *Stelospongos* I was also unable to detect the flagella, but I expressed my belief in their existence in the living sponge, and gave a diagram showing them. My researches on *Halichondria panicea* fully justify this view. Fig. 1, which is a careful drawing of an actual preparation, shows the flagella plainly, projecting from the bodies of the collared cells through the collars and into the cavity of the chamber (a). Thus the question as to the coexistence or otherwise of Sollas's membrane and the flagella of the collared cells is settled.

If we now look down upon the wall of a chamber from the outside, instead of examining it in section, another important point is brought to light. The collared cells, which, it will be remembered, stand well apart from one another, are connected at their bases by broad protoplasmic processes, so that the wall of the chamber appears to be made up of a network of broad protoplasmic strands with nuclei at the nodes of the net (fig.

1, *b*). This fact has also been observed by Sollas in the case of the *Tetractinellida* (op. cit.), and is well shown in his diagram of the protoplasmic continuity of a sponge. I have also seen and figured at *x*. in fig. 1 what appears to be a connection of a collared cell with a stellate cell in the surrounding mesoderm, as described by Sollas.

We have now to face the difficult question, "How does the water from the inhalant lacunæ enter the flagellated chambers?" Sollas (op. cit.) observes, "Since the fenestrated membrane stretches across the flagellated chamber transversely there must be an aperture or apertures in it for the passage of water from the prosodus to the aphodus, though I have never succeeded in finding any; it is possible that the apertures have the form of pores no larger than the lumen of the choanocytal collars, and in this case they would be very difficult to distinguish."

In spite of the excellent preservation of my sections I can find in *Halichondria panicea* no apertures in Sollas's membrane to allow of the passage of the water, and I am inclined to believe that either there are none, or that they exist merely as temporary and not definite openings, which is quite possible. The idea that there are no openings may seem at first sight rather strange, but I believe it to be quite reasonable, if not probable.

After having arrived at this point in the present paper I at length obtained access to Bidder's "Note on the Physiology of Sponges,"<sup>1</sup> which I had hitherto been unable to see. I find that this author has forestalled me in a suggestion which I was about to make concerning the function of Sollas's membrane, namely, that it serves to filter food particles from the current of water flowing through the sponge. As the note in question is very brief I may perhaps be allowed to quote it in full.

"After feeding with suspended carmine a calcareous sponge (*Leucandra aspera*, Vosmaer) the author found that in it the carmine was at no time in any but the collared cells. The

<sup>1</sup> 'Proceedings of the Cambridge Philosophical Society,' vol. vi, part 4 p. 183.



water is filtered of the particles suspended in it by a membrane, formed by the coalescence of the collars, which stretches completely across the current. This coalescence has been figured by Sollas in certain siliceous sponges. The whole evolution of the canal system in sponges consists in increasing the energy of the oscular flow and diminishing the velocity in the flagellate chambers. In these are alike specialised the functions of absorption and propulsion, since to each a low velocity is advantageous. The author believes that the collared cells primitively both ingest and digest the food, the collars having as their function its retention; digestion is only secondarily passed to the mesoderm."

It appears almost certain that in some sponges Sollas's membrane completely stretches across the prosopyle, so that the water has actually to pass through it on its way into the chamber. We may compare the passage of the water through Sollas's membrane to the passage of liquids through organic membranes by osmosis, only in sponges it is the flagella of the collared cells which supply the necessary motive power.

Nevertheless, although Sollas's membrane probably acts in all cases as a trap to catch food particles—a conclusion arrived at independently by Bidder and myself—yet it is not likely that it always stretches completely across the prosopyles or inhalant apertures of the chambers. Whether it does so or not probably depends upon the diameter of the prosopyles. Thus in the Sycons, such as *Grantia labyrinthica*,<sup>1</sup> with large prosopyles, we may safely assume that the membrane ceases around the margin of the prosopyle. In many cases it must, from the nature of the case, be impossible to tell with absolute certainty whether the membrane is continuous across the prosopyles or otherwise; for even if, as figured by myself in *Stelospongos*, a gap appears in the membrane, we can never be certain, in dealing with such a delicate structure, that the membrane has not been accidentally ruptured by the mode of preparation adopted. Again, it is quite possible and even probable that temporary gaps frequently make their

<sup>1</sup> Vide Dendy, "Studies on the Comparative Anatomy of Sponges," I II.



appearance in the membrane, which is, after all, only an extension of the delicate protoplasmic collars of the collared cells, and it is well known how plastic and changeable such structures are.

The main point, however, which I wish to establish concerning Sollas's membrane in the present article is its coexistence with the flagella of the collared cells, which has not hitherto been proved. I wish also to support Bidder's view that its function is the retention of food particles, which function would be most effectually fulfilled by its stretching completely across the prosopyles, and less so in cases where the prosopyles are too wide to admit of this.

I have now to describe the structure of the ova, which are remarkable for their great complexity. In my third study on the comparative anatomy of sponges I have described how the mature ova of *Grantia labyrinthica* migrate through the walls of the inhalant lacunæ and remain suspended therefrom, each by a distinct peduncle, awaiting fertilisation by the spermatozoa which enter the sponge with the incoming stream of water. This is probably the mode in which fertilisation is effected in most if not in all sponges. Fig. 2 represents a mature ovum of *Halichondria panicea* suspended by a peduncle from the wall of a lacuna. I have been unable to prove that the lacunæ in which I have found ova suspended in the case of *Halichondria* are inhalant, but from the analogy of *Grantia labyrinthica* we may assume that this is the case. The adult ovum is about 0.067 mm. in total diameter. Its outermost portion forms a distinct envelope around the ovum proper. The envelope (fig. 2, *env.*) is fairly thick, and projects at one side to form the peduncle (*ped.*), by means of which the ovum is attached to the wall of the lacuna. The substance of the envelope is but faintly granular, and stains lightly; the peduncle in the specimen figured appears to be hollow, but I should doubt if this is a constant character. Within the envelope the ovum proper is suspended as in a bag. It is spherical, about 0.053 mm. in diameter, uniformly and rather coarsely granular, and stains deeply with borax

carmine. In its centre is the nucleus, a spherical body invested by a very thick and distinct membrane, and measuring, together with its membrane, about 0·02 mm. in diameter. The substance of the nucleus itself is very finely granular, and stains but lightly. The nuclear membrane, however, stains deeply. There is also a single spherical nucleolus which stains very deeply, and is placed excentrically, touching the nuclear membrane.

I have found several ova exhibiting the structure here described; they appeared to be worth mentioning as showing to what a degree of complexity the ovum even of a sponge may attain, and also as affording some confirmation of my views as to the mechanism of fertilisation of the ovum in sponges generally.

The above remarks deal only with certain points in the structure of *Halichondria panicea*; for the benefit of the student I may add that a fairly complete literature of the species is to be found on p. 2 of the Report on the 'Challenger' Monaxonida.

## EXPLANATION OF PLATE V,

Illustrating Mr. Arthur Dendy's paper "Studies on the Comparative Anatomy of Sponges. IV.—On the Flagellated Chambers and Ova of *Halichondria panicea*."

FIG. 1.—Small portion of a section vertical to the surface. Zeiss, F, oc. 3. *a, b, c.* Different views of three flagellated chambers. *i. l.* Inhalant lacuna. *e. l.* Exhalant lacuna. *e. o. c.* Exhalant opening of a chamber. *sp.* Spicules. *x.* Protoplasmic processes connecting a collared cell with a stellate mesodermal cell (?). *s. m.* Sollas's membrane.

FIG. 2.—An ovum suspended in a lacuna. Zeiss, F, oc. 2. *env.* Envelope of the ovum. *n.* Nucleus. *n. m.* Nuclear membrane. *no.* Nucleolus. *ped.* Peduncle.

**On *Megascolex cæruleus*, Templeton, from Ceylon; together with a Theory of the Course of the Blood in Earthworms.**

By

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With Plates VI—IX.

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INTRODUCTION.

DURING the summer of 1889 I visited Ceylon, with the view of determining the relation of the Earthworm Fauna of the island to that of Southern India.

I obtained an introduction from the Madras Government, and express here my great indebtedness to them, as well as to Sir E. Noël Walker, Colonial Secretary, to H. W. Green, Esq., Director of Public Instruction (Ceylon), and to T. C. Huxley, Esq., of Peradeniya, for their assistance in facilitating my work. To Mr. Huxley I am specially indebted for all my specimens of *Megascolex cæruleus*.

I obtained no fewer than thirty-eight<sup>1</sup> different species of

<sup>1</sup> Of these thirty-eight species I have only found seven in India, and at the present moment I know of about twenty-nine Indian species which I did not

earthworms, twenty-three of which are species of *Perichætidae*, and with very few exceptions are species which I have not found in India. As a long time must certainly elapse before I shall be able to publish complete accounts of all these forms, and the general views to which their study has led me, I publish here a special account of some structural features of *Megascolex cœruleus*, especially the details of its circulatory system, which its great size has enabled me to work out. Where I have dealt with matters other than those relating to *Megascolex* I have, as a rule, embodied them in foot-notes.

#### HISTORICAL AND SYSTEMATIC.

There cannot be the slightest doubt but that the worm here described belongs to the same genus as the worm described by Beddard (1)<sup>1</sup> as *Pleurochæta Moseleyi*.

This author (2), after an examination of the type specimens of *M. cœruleus* in the British Museum and in Edinburgh, and subsequently to the publication of the paper above referred to, came to the conclusion that his genus *Pleurochæta* was identical with the genus *Megascolex* established in 1845 by Templeton (14). I am convinced that the species also are identical, and that the name *Pleurochæta Moseleyi* must be considered as cancelled. It is perfectly true that, owing to the loss of the "Memnon," by which ship Templeton despatched to England his original memoir, the existing description is very scanty; but on the one hand we have Beddard's examination of the type specimens, and on the other the worm is very large (the largest known *Perichæte*) and of a striking colour, and from numerous inquiries which I made on the spot is, it appears, well known to Europeans and natives, and believed to be the only worm of that size in the island.

The question of the identity of *M. cœruleus* with any of find in Ceylon, making the amazing total of about sixty-seven species; and I have no reason to suppose that the field is by any means exhausted. In the town of Madras there are four species: *Perichæta armata*, two new species of *Acanthodrilus*, and a minute species of *Moniligaster*.

<sup>1</sup> These numbers refer to the bibliography at the end of this paper.



Schmarda's species is much more difficult. That Schmarda himself was led away by a mistaken interpretation of Templeton's description has often been pointed out. Templeton wrote, "Each ring is dilated in the middle of its length into a ridge, which carries on it, except in the mesial line of the back, minute conical mammillæ, two in number, each surmounted by a minute bristle;" while Schmarda (13) speaks of "the ridges which in *Megascolex* were stated to be found only on the dorsal side, continued in our specimens also on to the ventral side."

It is just possible that the *Perichæta leucocycla* of Schmarda may be the same worm; I am unable to identify it with any other species I found in Ceylon.

As to the best use of Schmarda's generic term *Perichæta* I shall speak in a subsequent paper. I agree entirely with Rosa (11, A) in his classification of the *Perichætidae* as far as it goes, but am convinced that it will be necessary to create several other genera.

#### HABITAT.

All my specimens of *Megascolex cæruleus* came from the neighbourhood of Kandy and Peradeniya and the hills near, that is to say, from an elevation of about 1700 feet. I have no reliable information as to its occurrence at any higher or lower level. I was not fortunate enough to obtain a specimen myself, and so be enabled to note any peculiarities as to its habitat.

#### EXTERNAL CHARACTERS.

**Colour and Size.**—The general appearance, colour, and size may be judged from figs. 1 and 2.<sup>1</sup>

<sup>1</sup> I am indebted to my wife for coloured external views of all the worms which I have procured in a living state, and shall not give any further description. Probably no two observers would agree in describing the colour of an earth-worm, and an accurate figure of a full-grown living specimen gives a better idea of the size than any measurements. In no case can we rely absolutely upon measurements of length, as the worm is constantly changing its amount

The pigment changes very slightly, at any rate for some months, when the worm is preserved in spirit; but the reddish portions shown in the figures, which are due to absence of pigment and great blood supply, naturally disappear when the specimen is placed in spirit. When the worm is alive these red portions are very noticeable, and indicate the greatly increased blood supply in the prostomium and round the mouth, and in the neighbourhood of all the genital apertures (fig. 2).

Number of Segments.—I have counted the number of the segments in specimens varying from 10 inches to 32 inches in length, and have found it to vary between 250 and 290, being usually about 270. In fig. 1, 286 segments are shown.

Prostomium.—This is, as is often the case, of a blood-red colour, and is broad and blunt at the extremity; there is a short transverse groove along its line of junction with the peristomial (buccal) segment, and both it and the latter segment present in spirit-preserved specimens numerous longitudinal grooves.<sup>1</sup>

Setæ.—In shape the setæ are, as compared with those of many perichæte worms, short, stout, and only slightly curved.

As to their position, they are of course arranged in rings, of contraction, and a contraction which produces a considerable alteration in length produces a barely measurable increase of thickness.

It is interesting to note that worms of some species possess a much greater power of contraction than others. *P. mirabilis*, for instance, contracts and extends itself to but a very slight degree, and has a movement like a Nematoid, while *Moniligaster grandis* can contract itself to about a quarter of its fully extended length.

<sup>1</sup> We have at present few data to enable us to make much use of the prostomium in classification. It will be necessary to compare its structure in a large series of living worms. In preserved specimens it becomes contracted to a large and very variable extent. Moreover, it never has when the worm is alive the same shape for two seconds together. Many worms have a habit of constantly protruding a large portion of the pharyngeal region, and there is no line of demarcation between the prostomium on its ventral side and this latter region.

and there is a small gap in the ring in the median dorsal and ventral lines. The dorsal and ventral gaps are equal in the greater part of the body to the interspaces between three or four of the neighbouring setæ. The seta gaps are throughout smaller in the ventral than in the lateral and dorsal regions.

In the most anterior segments the gaps, especially in the lateral and dorsal regions, and above all the median dorsal gap, are much larger than in the posterior segments. The number of the setæ is greater than in any other recorded *Perichæta*. There are 120 to 140 in most segments, but considerably fewer in the most anterior segments. I have found as few as thirty-six in segment v.<sup>1</sup>

The most ventrally placed setæ, especially in the neighbourhood of the male pores, are a little larger than usual, but I should not speak of them as "modified" setæ. There are no setæ in segment XVIII between the male pores.<sup>2</sup>

I have never found the setæ absent from the clitellum, as

<sup>1</sup> I am in the habit of counting the setæ in segments v, ix, and xxv, the latter segment serving as a type for the rest of the body, and I find that the relation of these numbers to one another varies with other important characters rather than the actual numbers themselves.

The method I adopt in examining the arrangement of the setæ is to cut open the freshly killed worm on one side of the dorsal median line, to scrape out the viscera, to flatten the body-wall of the most anterior twenty-five segments between two glass slips, and to allow it to harden in spirit; subsequently to treat this piece of skin with potash followed by glycerine, and then to mount it.

This process renders it possible to see the follicles, even where the actual seta has fallen out.

The shape of the setæ varies so slightly in different species, that except in special cases it is of little use for classificatory purposes. In a preparation made as above, modified setæ in the neighbourhood of the spermathecal apertures or male genital pores should always be looked for.

<sup>2</sup> The presence or absence of setæ in segment XVIII between the male pores is a most important character among the *Perichætidæ*. In all worms which I think on other grounds it will be advisable to place in the genus *Perichæta* *s. str.* such setæ are present. They are also present in certain other worms which have other special peculiarities, but in a very large number of species, e.g. *P. armata*, and the majority of Fletcher's Australian species, they are absent.

recorded by Beddard (1), but then in none of my specimens was the clitellum very well developed.<sup>1</sup>

**Clitellum.**—The clitellum is not shown in my coloured figure, as I was unable to secure a live worm in which it was developed, but I have seen it in spirit specimens collected at some other time of year.

I have seen it developed upon the posterior portion of segment XIII, and upon every succeeding segment down to and including segment XXI; it is, however, always deficient ventrally from segment XVII onwards, so that the male pores do not actually open through clitellar substance.<sup>2</sup>

**Genital Apertures.**—The spermathecal apertures are placed between segments VII . VIII and VIII . IX, or rather just on the anterior margins (which is the usual arrangement) of the hinder of those segments in each case. They are all placed equally near the median ventral line, and about in a line with seta 9 (i. e. the ninth seta from the median ventral line).

The two oviducal apertures are placed very close together, and very slightly in front of the seta ring in segment XIV<sup>3</sup> (fig. 2).

<sup>1</sup> With regard to the presence or absence of setæ on the clitellum a good deal of unnecessary confusion exists. In all young perichæte worms which I have examined setæ are present on the clitellar segments, but when the clitellum develops they may remain projecting, or they may become buried in the clitellar substance, or any or all of them may actually drop out. I am inclined to think that all the species of one genus behave in the same way in this respect, but am not sure of this.

<sup>2</sup> I know of no perichæte worm in which the male pores do thus open. We shall have to distinguish between worms in which the clitellum forms a definite girdle, strictly limited in normal individuals to certain segments, and those in which it always shows a tendency to spread somewhat irregularly. Even if we abandon, as Vejdovsky (15) and Rosa (11, A) do, and as we certainly must, Perrier's classification (8) of earthworms into Antecitellians, Intracitellians, and Postcitellians, we may still use the character of the clitellum in classification.

<sup>3</sup> I know of no Perichæte in which there are any setæ between the oviducal pores; the latter always lie either in the ventral seta gap or slightly in front of the seta ring altogether. It is often a very difficult matter to ascertain whether the oviducal pores are paired or single and median.

I am now convinced that *P. saletensis* and *P. bivaginata*, which I



The male pores lie immediately ventrad of the most ventrally placed seta in segment XVIII. There are, as stated by Beddard, two other pairs of apertures in this region, though not exactly in the position stated by him. The one pair lies between segments XVII. XVIII, and the other between segments XVIII. XIX. These pores are the apertures of the glands described below (p. 83).

All these six apertures lie about equally near the median ventral line and opposite setæ 7 (figs. 2 and 13).

In the living worm there are no papillæ and no depressions in this region, but when the worm is killed and contraction of the body-wall takes place a depression is produced, as shown in fig. 13, and the pores lie at the edges of this depression, the male pores upon papillæ.<sup>1</sup>

**Dorsal Pores.**—The most anterior dorsal pore lies between segments VI and VII, and other dorsal pores occur between every two segments, including the clitellar segments.<sup>2</sup>

#### BODY-CAVITY.

This is as usual incompletely divided into separate chambers by the septa.<sup>3</sup>

separated chiefly because I believed them to differ in this respect, are the same species, and, indeed, the same as *P. armata*.

<sup>1</sup> There can be little doubt that the majority of papillæ which have been described in the region of the genital apertures of so many earthworms are not permanent structures, but produced by contractions of the body-wall; they are regions doubtless in which some portion of the muscular layers are absent, so that when general muscular contraction takes place they stand up as papillæ; and when a worm is so killed as to become greatly contracted these papillæ are seen, but if the same worm had been gradually killed they would not appear. Great care should consequently be exercised in using such papillæ as classificatory characters.

<sup>2</sup> These dorsal pores are important in classification. In some genera they are absent (*Eudrilus*, *Teleudrilus*, *Thamnodrilus*, *Moniligaster*, &c.). The position of the most anterior pore varies in different species. Sometimes they are absent from between every two clitellar segments.

<sup>3</sup> The body-cavity of any segment is ordinarily bounded by two septa. The anterior septum of a segment *N* may be conveniently termed septum *N* — 1. *N*,

The most anterior septum which is well marked is septum iv . v. I have not been able to satisfy myself as to the presence or absence of any septa anterior to this. The two or three most anterior septa are usually stated to be absent, but if present they are doubtless very thin and much obscured by the muscular fibres which radiate from the pharyngeal to the body-wall. I am inclined to think, both from dissection of this big worm and from sections of smaller worms, that these septa are usually present, and that the pharynx really lies in segment i.

Septa v . vi and vi . vii are very thin.

Septum vii . viii is also thin.

Septum viii . ix is thicker.

Septa ix . x, x . xi, xi . xii, xii . xiii are very thick.

The three succeeding septa are less so, and the septa behind these are all very thin. From all the thick septa arise numerous muscles, some of which are shown in fig. 3. These pass backwards through one or two succeeding septa to be attached to the body-wall.

These muscles, and indeed the thickened septa, must, as has been suggested, give greater strength to the anterior end of

and the posterior one septum  $n . n + 1$ . In stating that a certain organ lies in a certain segment great care is necessary with regard to two matters. In the first place, the septum may be attached to the body-wall at some spot more or less widely removed from the intersegmental groove. There is great variation in this matter in different genera; it is particularly noticeable in *Moniligaster*. In all such cases which I have examined by means of longitudinal sections the muscular fibres of the septum penetrate the body-wall at the right place, but have by a growth of connective tissue become adherent to the body-wall over some region behind the groove. In the second place, certain septa are at times undoubtedly absent or quite rudimentary. Perrier (9) states that two septa are absent in *Urochæta*. Beddard (3) also states that septum ix . x is almost absent in a species of this genus, and I have observed this to be the case in specimens of *Urochæta* obtained from both South India and Ceylon. The septa viii . ix and ix . x are absent, I believe, in all the species of one genus of *Perichætidae*.

The two or three most anterior septa are, moreover, absent in all worms, or so feebly developed as to have escaped notice, a fact which adds to the difficulty of enumeration.

the worm and assist it in burrowing. The conical shape of these anterior septa may be gathered from fig. 3.

#### ALIMENTARY TRACT.

I have little to add in this respect to Beddard's account for "Pleurochæta." I differ from him slightly with regard to the enumeration of the segments.

The arrangement of the anterior portion of the alimentary canal is shown in fig. 3.

The pharynx is bounded posteriorly by septum iv. v. This statement is not intended to exclude the possibility of there being thin, more anterior septa passing behind it. Numerous, doubtless unicellular glands lie outside on the walls of the pharynx, and open into it in the dorsal region.

The gizzard consists of an anterior thin-walled portion and a posterior thick-walled portion. Both portions are contained in the same segment. The former bears, projecting into its lumen, numerous hair-like processes which are lined by chitin and doubtless serve as a strainer. I am not aware that any such arrangement has been described in any other worm. The latter has a very thick muscular wall, and this is lined internally by thick, hard chitin. Following the gizzard is a narrow portion of the œsophagus, which extends from segment vi to ix.<sup>1</sup>

There is no specially large blood supply in the walls of the portion of the canal above described.

Segments x to xv contain the calciferous glands. In each of these segments the canal is swollen out into a bulb-like form, so that there is a series of such swellings, and between them in the region of the septa there are constrictions. I call these dilated portions of the œsophagus, calciferous glands, because there may usually be found in them smaller and larger concretions of carbonate of lime.<sup>2</sup>

<sup>1</sup> I use for the present the term œsophagus for any portion of the alimentary tract which lies anteriorly to the large intestine, and is not designated by any special name—as, for instance, pharynx or gizzard.

<sup>2</sup> I sent a number of these concretions to Professor W. D. Halliburton, of

The inner wall is much plicated, being raised into ridges and papillæ, and is excessively vascular.

The two most posteriorly placed glands are smaller than the other four.

It is beyond the scope of the present paper to discuss the relation of these glands to those found in other worms. I will merely remark that this is a very common type of calciferous gland among the Perichætidae, though by no means the only one. It is, moreover, the simplest.

Beddard mentions no such glands in "Pleurochæta."

In segments XVI and XVII there is a further portion of the narrow œsophagus, and in the latter of these this suddenly widens out into the intestine.

The intestine is, as Beddard has pointed out, for "Pleurochæta" very complicated.

The anterior portion may be called the typhlosolar region, and the posterior the post-typhlosolar region.

The typhlosole is in a very rudimentary condition. It is a mere ridge of the intestine projecting into the lumen along the median dorsal line. As stated below (pp. 61 and 70), there is no continuous longitudinal blood-vessel running along it, but the capillary network becomes so exceedingly dense here as to form a longitudinally placed blood lacuna.

This typhlosole extends as far back as segment CXXXV.

In the anterior portion of the typhlosolar region I find the intestinal wall forming the pouches described by Beddard (1, p. 492). In segments XVII to XXXV there are the large simple pouches, and in segments XXXVI to XLII are the seven pairs of sacculated pouches which correspond very closely to Beddard's description.

Posteriorly to these sacculated pouches the intestinal wall is not protruded to any great extent into pouches.

the Physiological Laboratory, University College, London, and am indebted to him for the information that they undoubtedly consist almost entirely of carbonate of lime. But he says, "A few shreds of organic matter remain undissolved. When the concretions are treated with acid, these prove to be of proteid nature."



In the post-typhlosolar region the intestinal wall becomes much thicker. In the hinder portion of the typhlosolar region lie the remarkable "kidney-shaped glands." I find twenty-two pairs of these glands, a pair in each segment from cxii to cxxxiii. Beddard describes only fifteen pairs, lying in segments lxxxvi to ci ("or thereabouts"). With regard to structure I can confirm in every particular Beddard's account. In my sections these glands present very much the appearance which Beddard figures, and each undoubtedly opens into the intestine. When the intestine is opened and its dorsal wall examined from the inside, the two rows of apertures are perfectly clearly seen. The glands in the middle of the series are the largest, and they get smaller and smaller at either end of the series. As I have stated elsewhere (circulatory system), the typhlosole, the anterior dorsal intestinal vessel, and the series of kidney-shaped glands all cease to exist at about the same spot.

I am unable to throw any further light upon the function of these glands.

I give no account of the histology of any portion of the alimentary tract. We still want information as to the histology of the alimentary canal in earthworms, but it should be in the form of a comparative account dealing with a series of typical genera.

#### VASCULAR SYSTEM.

It will be convenient to describe firstly the blood-vessels, and secondly the probable course of the blood-flow.

The red blood differs in no way from that fluid in other worms.

#### The Blood-vessels.

Dorsal Vessel (figs. 5 to 11, D.V.).—The dorsal vessel extends from the anterior portion of the pharynx to the last segment of the body. It lies above the alimentary canal, but is not adherent to the wall of the latter in any portion of its

course. Its walls are muscular throughout its length, but most so in segments VII to XIII, in which segments the vessel is much dilated. In the intersegmental (i. e. septal) regions, where the vessel is narrowest, there are in this portion of the vessel, and in all the portion posterior to it, valves which can doubtless entirely, or almost entirely, shut off the lumen of the vessel in each segment from that in the other segments. These valves are thick, semicircular ridges of connective tissue attached to the wall of the vessel across its ventral half, and presenting an irregular free edge (fig. 12).

I have never seen the dorsal vessel double in any portion of its course. I am surprised at this, as Beddard (1) distinctly states that in his *Pleurochæta Moseleyi* "it bifurcates no less than five times in the first eight segments, the bifurcations always coalescing again directly."<sup>1</sup>

Anteriorly the dorsal vessel bifurcates—that is to say, it gives off a pair of branches which behave in a manner similar to the other dorso-tegmentary vessels (see p. 71), and are to be considered as the most anterior pair of such vessels (fig. 8).

Posteriorly the dorsal vessel ends abruptly, as shown in fig. 11.

**Ventral Vessel** (figs. 4, 6 to 11, v.v.).—This is also known as the subintestinal or supra-neural vessel. Its walls chiefly consist of connective tissue, doubtless elastic, with circularly disposed bands of muscle in the regions of the septa. These bands would serve to secure a specially great blood-flow in the branches of the ventral vessel in any particular region of the body. It is of very uniform calibre throughout its entire length. At its anterior and posterior extremities it

<sup>1</sup> To prevent any confusion upon this point I may note that I am well acquainted with the "double" condition of the dorsal vessel which has now been several times recorded; it obtains in a perichæte worm which attains nearly the same size as *Megascolex cœruleus*, and which I discovered on the 24th May, 1887, on the Nilgiri Hills in South India. In this worm the dorsal vessel bifurcates in each segment from VII to XVI. I have noticed a sort of longitudinal folding in of the dorsal wall in certain segments of *M. cœruleus*, but the vessel is not double.

terminates by bifurcating, and thus giving off its most anterior and posterior branches, which are described under the heading *Ventro-tegumentary Vessels* (see p. 72).

*Supra-intestinal Vessels* (figs. 4 to 6).—There are, as Beddard states, two of these vessels lying side by side, but widely separated, on the wall of the œsophagus in segments IX to XIII. The two are joined by commissural vessels in segments X to XIII. In segment IX they lose themselves in the intestinal capillary network, and in segment XIV they join, and a very small median supra-intestinal vessel runs on into segment XVI, where it bifurcates and joins the dorso-intestinal vessels of that segment (fig. 5). It is not continued into the region of the intestine properly so called; there is consequently no typhlosolar trunk.<sup>1</sup>

The supra-intestinal vessels are, as usual, closely adherent to the intestinal (œsophageal, &c.) walls.

*Subneural and Latero-neural Vessels* are absent.<sup>2</sup>

*Intestino-tegumentary Vessels* (figs. 4, 6 to 10, i.r.).—This name was given by Perrier (9) to a pair of symmetrical longitudinal vessels which are connected at either end with a network of capillaries, the one network being in connection with some part of the œsophageal wall (i. e. some region in

<sup>1</sup> In many worms the supra-intestinal vessel is said to be prolonged backwards as the typhlosolar vessel. I should not be surprised to find that the typhlosolar vessel is much more frequently absent than is supposed, if not altogether so. Benham (4, p. 286) speaks of it as an "ill-defined vessel" in *Microchæta rappi*. Jaquet (6, p. 346) speaks of the circulation in the typhlosole as being "très difficile à poursuivre." Vejdovsky (15, p. 110) seems a little doubtful about it, and I have frequently seen in sections blood in that region which I should have said at once lay in the typhlosolar vessel, when further examination of the series of sections has convinced me that there was only a special development of the internal intestinal capillary network, a remnant doubtless of the sinus around the intestine of many *Chætopoda*.

<sup>2</sup> Lankester (7) calls the subneural vessel the ventral vessel, but I prefer to use this latter term for the subintestinal vessel, the latter being constant in the *Oligochæta*, while the subneural vessel is absent in all the simpler and many of the more complicated forms, e.g. many, if not all, *Perichætida*, *Pontodrilus*, and *Microchæta*.

front of the large intestine); the other network (set of networks it really is) being in connection with the pharyngeal wall, the septa, and the body-wall. Benham speaks of them as the "lateral longitudinal" vessels. I shall show that they are only the much-enlarged representatives in the anterior region of the body of a series of similar vessels which occur, a pair in every segment, in all the remaining portion of the body. There is, in fact, in *Megascolex*, and I expect in many other earthworms, a vessel on each side in every segment—in all except certain anteriorly placed modified segments—a vessel which communicates with capillaries at either extremity, the one network of capillaries being in connection with the intestinal wall, and the other with the body-wall, and the blood in circulating either passes from the intestinal capillaries laden with nutriment to the cutaneous and other capillaries for the nourishment of the tissues and for its own aëration, or is collected in an aërated condition from the cutaneous capillaries to pass to the intestinal capillaries for the absorption of nutritive matters.

A discussion as to which of these two courses it takes follows the description of the vessels. There is only a single pair of such vessels in the anterior modified region, and it is this pair of vessels which has been called intestino-tegumentary vessels by Perrier. I propose to apply the term intestino-tegumentary to the whole series, and to call anterior intestino-tegumentary vessels the large anteriorly placed modified pair, and intestino-tegumentary vessels of such and such a segment those in the posterior region.

The anterior intestino-tegumentary vessels have relations with the twenty or so most anterior segments, and are, I believe, in connection with the intestino-tegumentary vessels of the following segment, and these again with those that follow, and so on, by means of capillaries or very minute vessels. The relations of the large anterior pair have been dealt with by Perrier, Horst (5), Benham, Beddard, Jaquet, and others.<sup>1</sup>

<sup>1</sup> These vessels, or at any rate some vessels having somewhat similar relations, have been stated to communicate directly with the dorsal vessel in



I merely describe here what occurs in *Megascolex*, without any further detailed discussion of their views.<sup>1</sup> The main trunks of the anterior pair of intestino-tegumentary vessels run from the sides of the pharynx, lie freely in the body-cavity in the region of the gizzard, and then gradually take up a more ventral position, passing to the inside of the hearts, without being connected with them, till they become adherent to the ventral wall of the œsophageal (calciferous) glands.<sup>2</sup> The anterior extremity of each is connected with a network of capillaries on the pharyngeal wall. This network is connected with the network into which the most anterior branches of the dorsal and ventral vessels break up in this region (fig. 8). Passing backwards they are joined by various branches in the regions of the septa. An inspection of figs. 4 and 8 will show that these branches are segmentally arranged, and are connected with capillary networks on the septa and in the body-wall, which communicate on the one hand with either the ventro-tegumentary vessels or with branches of the most anterior heart, and so also with the ventral vessel. One specially large branch, which has also connections with the hearts of segments VI, VII, and VIII, communicates with a special network on the gizzard; another with the networks which are also connected with the four most anterior pairs of ventro-tegumentary vessels, and so on (see fig. 4). In segments X to XIII, i. e. those segments in which occur the four large œsophageal glands, the arrangement is somewhat different, and is almost exactly repeated in each of these segments. One branch is connected with the peripheral capillary networks, and two or three branches are connected with the

*Lumbricus*. In the absence of specimens of that genus I am unable to discuss this fact, nor its bearing upon my theory as to the course of the blood.

<sup>1</sup> It will be seen how little my account differs from that of Perrier, whose memoirs on *Urochæta* and *Pontodrilus* are most masterly pieces of work, and to whom I here express my many obligations.

<sup>2</sup> In fig. 4 this vessel is shown as lying at some distance below these glands. The drawing is diagrammatic to better elucidate the relations of the branches of this vessel.

"intestinal" networks, i. e. with those networks in the walls of the œsophageal glands.

The other intestino-tegumentary vessels have relations which are precisely similar to one another (figs. 4, 7, 9, 10). The main portion of the vessel in each case lies closely adherent to the body-wall just behind a septum, i. e. in the anterior portion of a segment; the ventral end of it is connected by several small branches (fig. 7) with the external intestinal capillary network, while from the trunk numerous branches, especially near the dorsal region, pass to the peripheral networks. The branches of the intestino-tegumentary vessels are thus of two kinds, peripheral branches and intestinal branches. All the intestino-tegumentary vessels place the peripheral and intestinal capillary networks in communication with one another, a relationship discovered by Perrier for the large anterior pair, which led him to give them the name of intestino-tegumentary vessels, and to compare them to portal vessels. The relationship, or existence even, of the posterior intestino-tegumentary vessels does not appear to have been hitherto described. They are connected with one another, the pair of one segment with that of the adjoining segments, and the one of one side with that of the other, in the intestinal wall, and, I believe, also in the body-wall (figs. 7 and 10). The longitudinal connections in the intestinal wall constitute doubtless the infra-intestinal vessel which is figured by Howes in the 'Atlas of Biology' (*Lumbricus*), and is stated by Benham (4, p. 253) to have been observed by Beddard in *Acanthodrilus*.

Hearts.<sup>1</sup>—There are eight pairs of rhythmically contractile

<sup>1</sup> I use this term for all rhythmically contractile, circularly disposed vessels, thus including, for reasons stated below, certain anterior branches of the dorsal vessel which do not join the ventral vessel.

These hearts are either—(1) all connected with the dorsal vessel, or (2) some only are so connected, while others are connected with the supra-intestinal vessel only, or (3) some are connected with the dorsal vessel only, and some with both the dorsal and supra-intestinal vessels (in *Pontodrilus* and *Titanus* [see Perrier, 10], in *Megascolides* [see Spencer, 13], in *Megascolex*, suggested as a possible arrangement by Beddard and figured in this

branches of the dorsal vessel; none of the other branches of the dorsal vessel, either those anterior or those posterior to these eight, are contractile.

The three anterior pairs lying in segments VI, VII, and VIII are lateral hearts; while the five posterior pairs lying in segments IX to XIII are latero-intestinal hearts, i.e. they are connected equally with the dorsal and the supra-intestinal vessels of their respective sides. An examination of fig. 5 will show that the dorso-intestinal vessels of segments XIV to XVI have similar connections, but they do not appear to be rhythmically contractile, nor have they the peculiar sphincter muscle at their distal extremities that the hearts have.<sup>1</sup>

The hearts of segment VI arise immediately in front of septum VI.VII (each pair of hearts arises immediately in front of the septum which divides the segment in which they lie from the segment next following). They extend only for a short distance on the wall of the gizzard, and terminate in a muscular bulb (figs. 4 and 6, *f*), a sphincter which is shut during the diastole, and opens at the systole of the heart. This heart is not connected with the ventral vessel. Two branches arise at its extremity, just beyond the muscular bulb. No branches arise directly from any of the hearts, i.e. between the point of connection with the dorsal vessel or dorsal and supra-intestinal vessels, as the case may be, and these muscular bulbs. The two branches above mentioned break up into capillaries on the walls of the gizzard, the network being connected, on the other hand, with branches of the anterior

memoir, and observed by myself in many other Perichætidæ). In all recorded cases in which there are some hearts which do not communicate alone with the dorsal vessel, such hearts are the posterior ones of the series.

I adopt Perrier's term lateral hearts for those which communicate with the dorsal vessel only, and his term intestinal hearts for those which communicate with the supra-intestinal vessel only, and shall use the term latero-intestinal hearts for those which have the dual connection to indicate that they correspond to both lateral and intestinal hearts.

<sup>1</sup> These peculiar relationships show how useless it is for classificatory purposes to record simply the number of pairs of "hearts," as is often done, without detailed account of what vessels are so named.

intestino-tegumentary vessels. The hinder branch comes into relation in this way with that branch of the anterior intestino-tegumentary vessel which lies on septum VI.VII.

The hearts of segment VII are of nearly twice the length of those of segment VI. They also give off two branches from the extremity beyond the muscular bulb; the one branch has relations similar to those described above with the branch of the intestino-tegumentary vessel which lies in septum VII.VIII, and the other joins the similar branch from the heart of segment VIII and goes to the gizzard, where it, like the corresponding branch of the heart of segment VI, is in communication by means of a capillary network with the intestino-tegumentary vessel.

The hearts of segment VIII are directly connected with the ventral vessel, but between the muscular bulb and this point of connection give off two branches, one of which is mentioned above, and the other has corresponding relations with the branch of the intestino-tegumentary vessel which lies on septum VIII.IX.

The five succeeding pairs of hearts are all latero-intestinal. Those of segment IX join the ventral vessel; but, as in the case of the hearts of the preceding segment, there is a certain length of vessel between the end of the heart, as marked by the muscular bulb and the ventral vessel; from this portion arises one branch only, which has the usual relations with a branch of the intestino-tegumentary vessel—that which lies on septum IX.X. There is no branch to the gizzard-wall; we are now behind that region. All these arrangements are shown in fig. 4.

All the segmentally arranged capillary networks with which the hearts and intestino-tegumentary vessels communicate, as described above, extend over the body-wall in their own neighbourhood.

The hearts of segments X—XIII have relations precisely similar to one another. Each is connected at its proximal extremity by a vessel much narrower than itself with the dorsal vessel, and by another narrow vessel with the supra-intestinal



vessel of its own side (figs. 4—6). These four pairs of hearts are much larger than any of the other hearts; they are monilated, and the muscular bulbs at their distal extremities are placed at the junction with the ventral vessel in each case; there are consequently no branches, for, as I have stated above, no branch ever arises from a heart proper. (Note in this connection the different behaviour of the intestino-tegumentary vessels in segments x to XIII; see fig. 4.)

Capillary Networks.<sup>1</sup>—It will be most convenient to consider these before speaking further of the vessels which put them in connection with the above-mentioned trunks.

We can, I think, recognise two groups of capillary networks only—peripheral networks and intestinal networks. I mean that there are no such things as special commissural networks placing any of the great trunks in communication with one another. If such special networks as have been described by various authors exist, placing the big longitudinal trunks in communication with one another, they may be suitably termed commissural networks. The most important of these which has been described is the network into which the dorsal vessel breaks up at the anterior extremity of the body, and which comes into relation with a similar network arising from the ventral vessel. But in *Megascolex*, at any rate, I see no reason why this should not be grouped with the peripheral networks. I wish, indeed, to bring into prominence the fact that we have, in the most anterior region of the body, only a series of segmentally arranged networks—nothing, in short, which differs from what obtains in other segments of the body. It must, however, be borne in mind that my investigations have been made upon a worm devoid of subneural and latero-neural vessels, the presence of which may entail other variations.

The networks which connect the dorsal vessel with the sub-

<sup>1</sup> Perrier (9, p. 466, foot-note) very justly points out that the term “capillary” is here used in a special sense, as there is nothing like the difference between a so-called capillary and a small vessel that obtains in a vertebrate animal; but the same remark applies with equal force to the use of the term in all animals other than vertebrates.

neural, described by Perrier (9) as occurring in segments VIII, X, and XIII, in *Urochæta*, may be special commissural networks.

Another capillary network, which, if it exists in any worm, would be a commissural network, is that connecting the dorsal and ventral vessels at their posterior extremities. From observations which I have made upon *Megascolex*, as well as upon some small worms, mounted alive in a compressorium, I believe that there is no such special network, but that the terminal branches of these vessels behave just like any other of their branches (fig. 11). Jaquet (6), however, speaking of *Lumbricus*, says that the dorsal and ventral vessels "se mettent en relation par des anastomoses réciproques" at their posterior extremities.

Peripheral Networks.—I would give this name to all the capillary networks in the skin in which the blood undergoes aëration, also those in the septa—in fact, all those which do not belong to the intestinal system; and I think we are justified in grouping all these together, as they all have similar relations with the large vessels, and are all metamERICALLY arranged.

In *Megascolex*, at any rate, these always establish communication between the dorsal, ventral, and intestino-tegumentary vessels. They lie for the most part in the superficial region of the body-wall, but they are also to be found in all the various tissues and viscera, excepting only the alimentary canal, and even here the exception does not extend to the pharyngeal and gizzard region. I have not had sufficient material to work out their exact arrangement in the nerve-cord, nephridia, generative organs, or walls of the large blood-vessels, in which latter they are to be found as *vasa vasorum*, but I am satisfied that in all these cases they have relations similar to those of the networks found in the body-wall. I believe that in all cases branches of the dorsal, ventral, and intestino-tegumentary vessels enter into connection with them. They are certainly continuous across the dorsal median line, and I believe also from segment to segment, though not of

course equally dense throughout, and it is therefore possible to speak of separate networks segmentally arranged.

The most anterior (fig. 8) is connected with the two most anterior branches of the dorsal vessel, those of the ventral vessel, and also the most anterior ramifications of the anterior intestino-tegumentary vessels.

Following these there is a series of similarly arranged networks throughout the body, the only exceptions being the slight ones about to be mentioned, and these are due to the presence of the hearts. In segments VIII to XIII the connections with the ventral vessel are wanting; in segments VI to IX there are no direct connections with the dorsal vessel, their place being taken by connections with the hearts of those segments, or rather with the vessels which are the distal connections of those hearts.

In all other segments there are connections with the dorsal vessel by means of dorso-tegumentary vessels; of the exact origin of these vessels in segments X to XIII I have no note. In all the segments as far back as XIII, and probably in the next four or five segments also, there are connections with anterior intestino-tegumentary vessels; in all the succeeding segments, and perhaps in the above-mentioned four or five segments, there are connections with the intestino-tegumentary vessels of the various segments.

**Intestinal Networks.**—All the capillary networks in the walls of the alimentary canal, excepting in its most anterior region, where the capillaries are more superficial and belong to the peripheral networks, fall naturally into one group, and are described below in detail for *Megascolex*.

These networks, as I have found them in *Megascolex*, differ considerably from those described as occurring in other genera. I select for detailed description one of the segments XLIII to CXXV (fig. 10).

There are two capillary networks in the alimentary canal wall—an internal deep-lying network and an external more superficial one.

The internal network (fig. 10), which corresponds to the

"quadrillage" of Perrier (9, p. 491) and Jaquet (6, p. 345), is so dense a network that it may be regarded as a blood sinus interrupted at certain spots; the interspaces are in certain places even smaller than the vessels which surround them. The meshes are not so regularly rectangular as they appear to be in some other genera; they are not equally so, moreover, in all regions of the intestinal wall. Near the typhlosole and also along the intersegmental lines the longitudinal portions of the network are specially developed, and the meshes fairly rectangular; in other regions they are less regular. The network is continuous from segment to segment, and across the dorsal and ventral median lines in each segment. The vessels are largest and the interspaces smallest over the typhlosolar region. There is indeed so much blood in this region that when the intestine is opened the typhlosole strikes the eye at once as a longitudinal band of red colour, noticeable even when the whole surface appears red. I have seen the vessels so distended that the interspaces were hardly recognisable. With the slight exceptions mentioned above, there is not very much difference in calibre among the vessels composing the network. All the vessels in any particular region may be distended or the reverse, but that is all. This internal network is directly connected with the dorso-intestinal vessels.

The external network is very different. The vessels are of very various calibres. There are large vessels which divide into small branches, and these subdivide and so on; the smallest branches form a complete network. These networks are arranged segmentally, and are not continuous, as networks, that in one segment with that in another, as is the case with the internal network. The network in each segment is continuous over the ventral median line, but not over the dorsal region. At very numerous spots little branches from the external network penetrate the intestinal wall and open into the internal network. The vessels of the external network have always clearly defined but very thin walls, do not seem capable of distension, and do not form anything like so close a network as the internal one. The vessels of the external network



give one the impression of an agency for distributing blood to the lacuna-like vessels of the internal network, as indeed, I believe, they are.

The external network is formed by branches of the intestino-tegumentary vessels.

In the hinder region of the intestine precisely similar arrangements obtain, but neither network is nearly so dense, and the internal network quite loses its lacunar character.

In the anterior region it is, owing to the pouches, difficult to make a flat preparation, and so to see these networks, but from what I have seen I conclude that the differences between the arrangements obtaining in this region and those above described are only slight.

**Dorso-tegumentary Vessels** (figs. 4 to 7, 9, D. T.).—These are branches of the dorsal vessel connecting it with the peripheral networks. In *Lumbricus*, according to Jaquet (6), each divides into a “branche tegumentaire” and a “branche dorso-sous-nervien,” which latter is connected with the nephridial capillaries. According to Perrier (9) a similar arrangement obtains in *Urochæta*; while in *Pontodrilus*, which is devoid of a subneural vessel, the “branche dorso-sous-nervien” is suppressed; this is also the case in *Megascolex*.

All the branches of the dorsal vessel anterior to the hearts, and one pair of those branches in each segment posterior to them, belong to this category.

In the most anterior portion of the dorsal vessel they arise from this latter slightly irregularly, i.e. unsymmetrically (fig. 8).

In the region of the three pairs of lateral hearts and the most anterior pair of latero-intestinal hearts there are no such vessels.

In the region of the four posterior pairs of latero-intestinal hearts there are such branches, but I have no note as to their exact place of origin. It may be some months before I obtain any fresh material, and it is a point of such minor importance that I leave it undetermined.

In all other segments they arise, regularly, from the dorsal

vessel immediately posteriorly to the septum which forms the anterior boundary of the segment in which they lie.

**Ventro-tegumentary Vessels** (figs. 4, 7 to 9, v. t.).—These are branches of the ventral vessel connecting it with the peripheral network. There is a pair of these vessels in every segment of the body except the first, in which there is a branch of that belonging to the second segment (fig. 8) on each side, and except in those segments in which the ventral vessel is joined by hearts, viz. VIII to XIII.

As mentioned above, the hearts of segments VI and VII do not join the ventral vessel, and in these segments there is, as usual, a pair of ventro-tegumentary vessels.

In the last segment of the body the ventral vessel simply comes to an end by giving off two of these branches (fig. 11).

**Dorso-intestinal Vessels** (figs. 4 to 7, 10, d. i.).—These are branches of the dorsal vessel placing it in connection with the intestinal capillary networks. In segments I to IX there are no such vessels. In segments X to XIII their place is taken by vessels opening into the supra-intestinal vessels, of which there are two pairs in each segment. These may be called supra-intestino-intestinal vessels. In segments XIV to XVI there is a single pair in each segment, connected, as are the latero-intestinal hearts, with both the dorsal and supra-intestinal vessel (see fig. 5). In segments XVII to CXXXV there are two pairs in each segment, the anterior one being always smaller than the posterior (fig. 10). In segment CXXXVI and the following segments to the end of the worm there is only a single pair in each segment, the pair which corresponds to the posterior pair of segments possessing two pairs.

These dorso-intestinal vessels are usually covered by the yellowish-brown coelomic epithelium cells which are so constantly found in the dorsal region of the alimentary canal.

The vessels in the segments anterior to the large intestine soon penetrate the intestinal wall; of those in the region of the large intestine, where there are two to the segment, the anterior one always passes round to the ventral region before penetrating the wall, while the posterior one, after having

received a number of little branches (fig. 7), soon penetrates the intestinal wall. After penetrating the wall both vessels pass on towards the ventral line, receiving numerous branches from the internal capillary network.

All these dorso-intestinal vessels are formed by the lacunar network, the anterior pair rather nearer the ventral median line than the posterior pair (fig. 10).

I have now described all the important vessels in *Megascolex*.<sup>1</sup>

#### COURSE OF THE BLOOD.

Having described all the principal vessels and their relations with one another, I shall now discuss the probable course of the blood, and put forward a theory which is on the whole simpler than any which has been hitherto propounded.

The reader should bear in mind throughout the description that, according to my theory, so long as the modified anterior extremity, about the first twenty segments, remain intact, and the thereby injured extremities of the longitudinal vessels shrink so as to prevent bleeding, it is possible to remove any or all of the succeeding segments without interfering at all with the circulation—a most important condition of any tenable theory, and, moreover, a state of things which indicates the metamerically segmented character of the vascular system, always excepting the cephalisation in the anterior region.<sup>2</sup>

There appears to be entire agreement among previous observers as to two points only—the forward direction of the

<sup>1</sup> Other special vessels have been described in various genera. In worms possessing a subneural trunk there are of course branches connected with it. These branches connect it, I believe, directly with the dorsal vessel, and indirectly with the intestino-tegumentary vessels (see Jaquet, 6). Where large nephridia occur the capillary network in connection with them would come under the group of peripheral networks, and communicate with branches of the ventro-tegumentary vessels on the one hand, and with branches from the subneural or (? and) intestino-tegumentary vessels on the other.

<sup>2</sup> I shall use the term cephalised region in the succeeding paragraphs as designating that region of the body in which the vascular apparatus is not similarly repeated in each segment.

blood-current in the dorsal vessel, and the downward direction of that in the hearts.

Of the blood which is brought forwards to the cephalised region in the dorsal vessel the greater portion goes into the hearts. Further, some or all of the blood going to some of the hearts may be derived from the supra-intestinal vessels—some in worms possessing both lateral and latero-intestinal hearts, all in worms possessing both lateral and intestinal hearts; the other and most anterior hearts receive all their blood from the dorsal vessel.

When we come to the questions—1. Whence comes the blood into the dorsal vessel? 2. Does any blood leave the dorsal vessel other than in the cephalised region?—we find great difference of opinion. There is in *Megascolex*, and probably in other worms, no great inflow at the posterior extremity; and when the dorsal vessel is filling, it fills simultaneously along the greater part of its length. According to Perrier (9, p. 504) and Benham (4, p. 255), blood enters the dorsal vessel from what I term the dorso-tegumentary vessels. I do not believe this to be the case. Vejdovsky (15, p. 115) states, though without giving any reason, that the blood flows from the intestinal capillaries into the dorsal vessel, and in this I agree with him.

The problem may be stated as follows:

A large quantity of blood leaves the dorsal vessel in the cephalised region. In some worms some of that blood may come to the dorsal vessel, in that region, from the supra-intestinal vessels, i. e. from the intestinal capillaries; but what we want to determine is, does any of it come in all worms from branches connected with the dorsal vessel in the posterior regions of the body? The vessels which are so connected seem always to be of two kinds, dorso-tegumentary vessels and dorso-intestinal vessels; and there can be, I think, no doubt that, in order to constantly replenish the dorsal vessel along its whole length, blood must come from one of these two kinds of vessels. Does it come from both kinds, or only from one? If the latter, from which? I think from the dorso-intestinal



vessels only. Perrier (9, p. 504) having observed the dorso-intestinal vessels full when the dorso-tegumentary vessels were empty, and vice versâ, comes to the conclusion that these two kinds of vessels play opposite rôles, and decided on other grounds that the blood flows from the dorsal vessel to the intestinal capillaries, and towards the dorsal vessel in the dorso-tegumentary vessels. Benham (4, p. 283), following in Perrier's footsteps, brought forward the arrangement of the valves at the points of junction of these various vessels with the dorsal vessel in *Microchæta* in support of the theory; while Vejdovsky, as I have stated above, takes the opposite view with regard to the direction of the blood-flow in the dorso-intestinal vessels at any rate.

I can confirm Perrier's observations that the two kinds of vessels in question play opposite rôles. I have made this observation in several recently killed and opened worms of large size, and also in small worms mounted whole in a live-box; further, in a large *Megascolex* recently killed and opened I have emptied all these various vessels in one region by gentle pressure with the finger, and then watched them refill themselves; and, moreover, I have cut a vessel of each kind and watched to discover from which of the cut ends blood flows; and, lastly, I describe below an arrangement of valves slightly different from that described by Benham for *Microchæta*, and having, I believe, an exactly opposite function.

Observations made in all these various ways have convinced me that the dorso-intestinal and dorso-tegumentary vessels do play opposite rôles, but in the reverse way to that imagined by Perrier.

The blood enters the dorsal vessel in each posterior segment through dorso-intestinal vessels, and leaves it by dorso-tegumentary vessels. The single pair of dorso-tegumentary vessels are always small as compared with the dorso-intestinal vessels, and there are in many worms (e. g. *Megascolex*) two pairs of these latter in many segments, so that doubtless more blood enters the dorsal vessel than leaves it in each posterior segment, and the excess is passed forward to be sent out in the

cephalised region. With regard to the arrangement of valves, in *Megascolex* (fig. 12) there are valves, as Perrier has described, in the dorsal vessel between every two segments, and there is also a valve at the junction of each dorso-intestinal vessel with the dorsal vessel. These latter valves consist of a soft-looking tissue which projects as a circular ridge into the dorsal vessel, and in the centre of this is the aperture of the vessel, and when the walls of the dorsal vessel contract the effect must be to occlude the apertures of the dorso-intestinal vessels. The dorso-tegumentary vessels present no such valves at their openings into the dorsal vessel, and are placed, moreover, in the anterior portion of each segment of the dorsal vessel just posterior to the valve lying in the dorsal vessel itself, so that the effect of a forward peristaltic contraction of the latter must be to force blood into these dorso-tegumentary vessels.

I cannot help thinking that the arrangement of valves described by Benham (4, p. 283) for *Microchæta* needs confirmation. His theory that such a valve as he describes at the entrance to each dorso-intestinal vessel serves to direct the blood into that vessel does not seem to me to be based upon sound hydrodynamical principles.

Two additional sets of facts—the relations of the two sets of intestinal capillaries to one another, and the relations of the intestino-tegumentary vessels—lend support to my view as to the direction of the blood in the branches of the dorsal vessel; and, moreover, as there is no doubt that in a certain region blood flows from the intestinal capillaries into the supra-intestinal vessel or vessels, and so into the dorsal vessel or into some of the hearts, the opposite view presents this anomaly, that the direction of the blood-flow in the intestinal vessels varies according to it in different regions; in other words, that some of the dorso-intestinal (or it may be supra-intestino-intestinal) vessels are efferent intestinal vessels and others afferent; according to my view they are all efferent, and further, the flow of blood always takes place from the dorsal vessel to the various peripheral networks, both in the

region of the body in front of the hearts and in that behind them.

The ventral vessel has two kinds of vessels connected with it—hearts and ventro-tegumentary vessels—which communicate with peripheral capillary networks.

The theory with regard to the flow of blood in the ventral vessel universally current is that it flows backwards along its whole length. I do not believe that this is the case. By far the largest amount of (I believe the only) blood coming into the ventral vessel comes through the hearts, and enters, owing to the forcible contraction of the latter, at considerable pressure. Why should it flow backwards only? What pressure can there be in the anterior portion of the ventral vessel to resist any flow in that direction? The only pressure which would tend to have this effect would be caused by the flow of blood from the anterior branches of the dorsal vessel; and if this blood flow into the ventral vessel it is probable that there is not a sufficient quantity of it to fill also the intestino-tegumentary vessels, and that the blood in these vessels also flows forwards. There are other reasons which render this unlikely; but even supposing it were the case, we have on the one hand pressure caused by the flow of blood from the dorsal vessel, which passes through the dorso-tegumentary branches and through peripheral capillary networks, and added to this pressure caused by the flow of blood which has (according to the assumption) passed through intestinal capillaries, through the intestino-tegumentary trunks, and finally through peripheral networks; while on the other hand we have pressure caused by the simultaneous contraction of all the largest and most powerful hearts. There can be no doubt which of these two pressures would be the greater—the latter. I conclude, therefore, that with regard to the ventral vessel, all the blood which enters it comes from the hearts, and that all the ventro-tegumentary branches—those anterior to the hearts, as well as those posterior to them—are efferent vessels. So far as the ventral vessel itself is concerned, they carry blood away from it.

We must now consider the intestino-tegumentary vessels, and we shall find that the conclusion we have just arrived at simplifies matters enormously with regard to these vessels. There has hitherto existed considerable uncertainty as to the direction taken by the blood in these vessels. Perrier (9, pp. 496, &c.), after an admirable discussion of the subject, in which he was evidently tempted, on account of the connection between the capillaries at the anterior extremities of the only pair of these vessels known to him and those at the anterior extremity of the dorsal vessel, to believe that there was in the vessels in question a backward flow, a conclusion to which he afterwards came for *Pontodrilus*, states that the blood flows forwards in them, in *Urochæta*. If, as I have asserted on independent grounds, the blood flows forwards in the portion of the ventral vessel anterior to the hearts, all difficulty disappears, the blood flows from the dorso-tegumentary and ventro-tegumentary vessels into peripheral networks, and from these into the intestino-tegumentary vessels, and from these again into the intestinal capillary networks. So that the afferent vessels of these latter are the intestino-tegumentary vessels, which accords with the theory that I have advocated above that the dorso-intestinal vessels are their efferent vessels. These arrangements obtain not merely with respect to the large anterior pair of intestino-tegumentary vessels of the cephalised region, but also with respect to those which occur in every other segment of the body. Yet one more point with regard to the subject of the preceding paragraphs. According to previous theories the blood at the anterior region in such a worm as *Megascolex* either flows forwards in three trunks and backwards in one, or backwards in three trunks and forward in one; if what I have said is true, the blood flows forwards in two trunks and backwards in the other two, which, as all four trunks are of approximately the same calibre when filled, seems a more probable arrangement.

Further, a reference to the various memoirs will show what doubt there has always been as to any special peripharyngeal vessel, i. e. pair of commissural vessels uniting the dorsal and



ventral vessels at their anterior extremities. The existence of a peripharyngeal vessel would be, of course, inimical to my theory. Even Jaquet (6, p. 340, and fig. 35), who asserts its existence, figures it as becoming capillary at one portion of its course. I am certain that it does not exist in *Megascolex*; in fact, the capillaries of the most anterior branches of the dorsal and ventral vessels are not connected from a functional point of view with one another, but all with those of the intestino-tegumentary vessels.

It follows from what I have said that, with regard to the capillary networks, the afferent vessels of the peripheral networks are in all cases branches of the dorsal and ventral vessels, while their efferent vessels are branches of intestino-tegumentary vessels, and the afferent branches of the intestinal networks are branches of the intestino-tegumentary trunks, and their efferent vessels are branches of either the typhlosolar, the supra-intestinal, or the dorsal vessel, so that blood coming from them is driven either into the hearts or into the dorsal vessel at its anterior extremity, and thus in either case into peripheral networks, so into the intestino-tegumentary system, and once more into the intestinal capillaries.

Every observation which I have made in *Megascolex* tends to bear out this theory of the circulation. The theory has, as I have implied above, this undoubted merit, that it exhibits the vascular system as a perfectly metamerically segmented organ, that portion of it contained in the cephalized region representing, as a whole, almost exactly the portion contained in any other segment of the body; the former has undergone, in fact, a synthesis, and certain additional structures, the hearts, have become developed in this region.

I may add a word or two about certain special points.

The narrow portions which join the heart to the dorsal or supra-intestinal vessels, or to both, found in so many worms, possess no little interest. In the most complicated case, that of a latero-intestinal heart, blood flows into the heart from the dorsal and from the supra-intestinal vessel, and fills it; the muscles at the point where the heart swells then act like a

sphincter (they appear to have such a structure), and when the heart contracts blood cannot flow back into either vessel. Again, the muscular bulbs at the distal extremities of the hearts probably act like sphincters, and ensure distension of the hearts during their diastole, so that the systole has greater effect; and after the systole their contraction prevents regurgitation from the ventral vessel. Again, it is interesting to recall here what is stated above with regard to the intestinal capillary networks. Blood flows from the external network into the lacunar spaces, forming the internal network, and at very low pressure—a circumstance favorable, doubtless, to intestinal absorption; thence it drains away gradually into the dorsal vessel; indeed, I expect that there is some slight pumping action exerted by the latter. Moreover, the anatomical arrangements of these intestinal networks indicate in some slight degree the probable direction of the blood-flow; the external network looks like an agency for distributing the blood to the lacunæ, and in so far as this is the case it bears out what is stated above with regard to the direction of the flow.

The peripheral networks deserve a special note in respect of their triple connections. They are always supplied with blood by two vessels in which there is some pressure, and so the blood is pushed on into the third, which is always some branch of an intestino-tegumentary vessel, and which thus always conveys the blood to the intestinal wall. The arrangement of such vessels as those shown in fig. 4, *g*, passing from periphery networks in the region of the calciferous glands to the intestino-tegumentary vessels, once struck me as presenting a little difficulty. I mean that, as it must according to my theory, blood should be flowing towards the main trunk of the intestino-tegumentary vessel in them, whilst it is flowing away from it in branches which open close by them and go to the intestine. But it must be remembered that the whole question is one of relative pressure. In no part of the intestino-tegumentary system can the pressure be very great, as it is only connected with the contractile vessels by means of capillaries; but the vessels in question form part of a set of branches through

which blood is entering the intestino-tegumentary system at a certain pressure, which, however slight, would be greater than that in the intestinal capillaries; into these latter the blood would consequently flow.

Summary.—The vascular system consists of a portion in the cephalized region, and of other portions metamerically repeated in all succeeding segments.

The cephalized portion differs only from that occurring in any other segment in having undergone a synthesis, and also in the presence of contractile hearts.

Throughout the body blood is forced from the contractile vessels into peripheral networks; thence it is conveyed by a system of intestino-tegumentary vessels to intestinal capillaries, and from these it returns to the contractile vessels.<sup>1</sup>

#### NEPHRIDIA.

Nephridia are present in the form of minute scattered tubules, and may be seen over almost the entire extent of the body-wall. There are no large tufts of tubules.

I have not at present worked out the structure of these Nephridia; they present peculiar difficulties in that they are most minute (actually smaller than in any *Perichæte* known to me), and at the same time the body-wall, as might be expected from the great size of the worm, is exceedingly thick.

<sup>1</sup> The theory, based upon observations on *Megascolex*, which I have put forward with regard to the circulation, brings us to such a plausible generalisation, and is borne out by so many structural details, that I cannot help thinking it will be found to have a very general bearing among earthworms, and it may be worth while to speculate for a moment as to the position in the scheme of the subneural vessels possessed by so many worms. So far as I can gather from the descriptions of the relations of such vessels they are in direct connection with the system of contractile vessels, and probably in indirect connection by means of peripheral networks with intestino-tegumentary vessels (see Jaquet, *Œ*, p. 340). In this case blood passes into them from the contractile vessels, and ultimately finds its way into intestino-tegumentary vessels and thence to the intestinal capillaries, and they do not thus affect the generalisations made above.

## NERVOUS SYSTEM.

I have nothing to add to Beddard's account of this structure. We have at present very few criteria for any useful comparison of the nervous system in different worms. It is, however, well known that there is less variation in the nervous system as it occurs in various worms than in almost any other system of organs. This fact—the constancy in structure of the nervous system in groups where most other organs vary greatly—so marked among earthworms and leeches, for instance, and so different from what occurs in planarians and nemertines, will doubtless acquire great interest when we know more as to the causes of variation in these lower groups of animals.

## GENERATIVE SYSTEM.

It would be going altogether beyond the scope of this paper to discuss in any detail the relation of this system as it occurs in *Megascolex* to that of other earthworms. I give a brief account only of the arrangements obtaining in *Megascolex*.

As the only points in which the structures in question differ greatly from those described by Beddard in "*Pleurochæta*" are such as anyone acquainted with the additions which have been made to our knowledge of this system of organs since the publication of Beddard's paper would have predicted, I shall not compare my account with Beddard's in detail.

Testes.—The testes (fig. 3) occur in segments x and xi, and are attached to the septa bounding these segments anteriorly, as is usually the case.

Seminal Funnel.—The seminal funnels lie of course in the same segments, and are attached to the septa bounding these segments posteriorly.

Vasa Deferentia.—The vasa deferentia, which are of course connected with the funnels, are exceedingly minute. The two on each side soon join together, and, running backwards, embedded in the longitudinal muscular layer, open into the muscular duct of the prostatic gland on each side, close to



where this latter opens to the exterior. They are ciliated internally.

**Prostates.**—The prostates are small relatively to the size of the worm and very compact, and each is provided with a very short muscular duct.

**Seminal Reservoirs.**—There is a single pair of seminal reservoirs (fig. 3) which lie in segment XII. It is very unusual to find a single pair only among the *Perichætidæ*.

**Ovaries.**—The ovaries lie as usual in segment XIII, attached to the anterior septum bounding that segment. They are very large.

**Oviducts.**—The oviducts are very small, and open internally in segment XIII near the nerve-cord; they then pass through septum XIII.XIV and penetrate the body-wall, and open to the exterior by the pores described above in segment XIV.

**Spermathecae.**—There are two pairs of spermathecae which are of the same size and shape. They are pear-shaped, and lie in segments VIII and IX.<sup>1</sup> Each possesses a small cæcum, which might entirely escape observation in a dissection, as it is completely embedded in the wall of the spermatheca itself at its basal region. It is very obvious in sections, and contains spermatozoa, while the spermatheca itself is empty in my specimen. The existence of these cæcal diverticula bears out the views recently expressed by Beddard on this subject.

**Accessory Glands.**—There only remain to be mentioned the two pairs of small glands which open between segments XVII and XVIII and XVIII and XIX.

These I have ascertained to be small solid glands composed of clear-looking cells which are not stained by borax-carmin.

<sup>1</sup> There has occasionally been some confusion as to the segment in which the spermathecae lie. They usually open in the groove between two segments and belong to the posterior of these two segments, but the septum is deficient just here, and they may occasionally be found pushed forward into the anterior of the two segments; but whenever I have found such to be the case I have pulled them back through the aperture in the septum, and it has become evident that they really belong to the more posterior segment.

These glands do not lie in the body-cavity, but are embedded in the muscular wall of the body in the spots where they severally open to the exterior. No trace of them can therefore, as Beddard says, be seen in an ordinary dissection of the worm. I cannot say what their function may be.

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## EXPLANATION OF PLATES VI—IX,

Illustrating Professor A. G. Bourne's Memoir "On *Megascolex cæruleus*, Templeton, from Ceylon; together with a Theory of the Course of the Blood in Earth-worms."

*Reference Letters to Blood-vessels.*

*D. V.* Dorsal vessel. *V. V.* Ventral vessel. *I. T.* Intestino-tegumentary vessels. *D. T.* Dorso-tegumentary vessels. *D. I.* Dorso-intestinal vessels. *V. T.* Vento-tegumentary vessels. *H.* Hearts. *per. cap.* Peripheral capillary networks. *int. cap.* Intestinal capillary networks. *S. I.* Supra-intestinal vessels. *S. I. I.* Supra-intestino-intestinal vessels. *a.* Vessels passing from a supra-intestinal vessel to a heart. *b.* Vessels passing from the dorsal vessel to a heart. *c.* Commissural vessels joining the two supra-intestinal vessels. *d.* Anterior extremities of the supra-intestinal vessels. *e.* Posterior extremity of the supra-intestinal vessels. *f.* Muscular bulbs at the distal extremities of the hearts. *g.* Branches joining intestino-tegumentary vessel, with periphery capillary networks. *h.* Vessels from the intestinal capillary networks joining dorso-intestinal vessels. *j.* Branches joining intestino-tegumentary vessels, with intestinal capillary networks. *k.* Vessel in the intestinal wall (infra-intestinal), passing from the intestino-tegumentary system of one segment to that of another. *l.* Origin of dorso-intestinal vessels from intestinal capillary networks.

## PLATE VI.

FIG. 1.—The entire worm drawn from life by Mrs. A. G. Bourne. Natural size.

FIG. 2.—Ventral view of the twenty-three most anterior segments. *m.* Mouth. *sp.*<sup>1</sup> and *sp.*<sup>2</sup> Spermathecopores (VII. VIII and VIII. IX). *ov.* Oviducal pores. ♂. Male pores. *gl.*<sup>1</sup> and *gl.*<sup>2</sup> Apertures of accessory glands.

## PLATE VII.

FIG. 3.—The anterior portion of the worm dissected from the dorsal surface. The alimentary tract is represented in horizontal longitudinal section. The septa have been cut in such a way as to render visible as much of the organisation as possible. Some of the generative organs are shown on the one side, and some on the other. The segments are numbered I—XIX. *m* Mouth. *ph.* Pharynx. *giz.*<sup>1</sup> Anterior portion of the gizzard, with straining

hairs. *giz.* Posterior portion of the gizzard, showing the thick muscular and chitinous walls. *ca. gl.*<sup>1</sup> The most anterior calciferous gland. *ca. gl.*<sup>6</sup> The most posterior calciferous gland. *int.* Intestine. *m. rad.* Radiating muscle. *t.*<sup>1</sup> and *t.*<sup>2</sup> The anterior and posterior testes of the left side. These have been completely pulled away from the septal wall to render them visible. *fun.*<sup>1</sup> and *fun.*<sup>2</sup> The seminal funnels corresponding to the two testes drawn. *sem. res.* Seminal reservoir. *ov.* Ovary. *pr.* prostate.

### PLATE VIII.

FIG. 4.—Slightly diagrammatic side view of the blood-vessels of the anterior portion of the body. The intestino-tegumentary vessels and their branches are represented by a dark colour in this and the succeeding figures in which they occur, the other vessels by lighter colour. The greater portions of the hearts in Segments x—xiii have been removed. The numbers i—xiii are placed close to the branches of the dorsal vessel belonging to those segments respectively. The lines numbered iv . v—xviii . xix mark fairly exactly the position of the septa at the level of the vessels drawn. Portions of the alimentary canal are marked as in fig. 3. The various peripheral networks are shown diagrammatically. *a.* Vessel passing from the supra-intestinal vessel to a heart. *b.* Vessel passing from the dorsal vessel to a heart. *f. f.* Muscular bulbs at the distal extremities of the hearts. *g. g.* Branches of the intestino-tegumentary vessels bringing blood from peripheral networks. *D. I.*<sup>1</sup> Anterior, and *D. I.*<sup>2</sup> posterior dorso-intestinal vessel of one segment.

FIG. 5.—View from the dorsal side of portions of the dorsal vessel and its connections in Segments vi—xviii. The dorsal vessel is shown cut in several places, and the cut ends turned backwards. The hearts and dorso-tegumentary vessels are shown cut short. *a. b.* As in fig. 4. *c.* Commissural vessels joining the two supra-intestinal vessels. *d.* Anterior termination of the supra-intestinal vessels. *e.* Posterior termination of the supra-intestinal vessels.

### PLATE IX.

FIG. 6.—Diagram of the vessels in a segment containing a calciferous gland. With regard to the origin of the dorso-tegumentary vessel in this region, see p. 023. *B. W.* Body-wall. *B. C.* Body-cavity. Other letters as before.

FIG. 7.—Diagram of the vessels in a segment in the typhlosolar region. The arrows denote the direction of the blood flow. *I. W.* Intestinal wall. *typh.* Typhlosole. *INT.* Intestine. *h.* Vessels from the intestinal capillaries joining the dorso-intestinal vessel. *j.* Vessels passing from the main intes-



tino-tegumentary vessel to the external intestinal capillaries. *int. cap.* Intestinal capillaries. Other letters as before.

FIG. 8.—Vessels at the anterior extremity of the body. The dorsal and ventral vessels are shown, but their branches are shown on one side or the other only. The intestino-tegumentary vessels are shown on the left side only. *cer.* Cerebral ganglia. *N.* Nerve-cord. The peripheral capillary networks belonging to the first five segments are diagrammatically shown and numbered. *per. cap. 1—per. cap. 5.* The branches of the dorsal vessel (dorso-tegumentary vessels) are numbered *i—v.* The septa are indicated by a line, and marked *iv. v* and *v. vi.*

FIG. 9.—Slightly diagrammatic view of the vessels in the body-wall, which is supposed to have been laid open by a cut a little to one side of the dorsal median line. The object of this figure is to show the relations of the peripheral networks. *j. j.* are the branches of the intestino-tegumentary vessels which pass to the intestinal wall. A comparison of this figure with figs. 7 and 10 will show the whole course and distribution of one of the posterior intestino-tegumentary vessels.

FIG. 10.—A view from the inside of a portion of the intestinal wall laid open by a lateral cut to show the intestinal capillaries. The dark coloured vessels and capillaries are connected by the branch marked *j.*, and other similar branches not shown, with the intestino-tegumentary vessel. These branches are supposed to be seen through by transparency; they really lie on the outside of the intestinal wall. Both capillary networks are in reality a little finer and denser than shown in the figure. The origin of the two pairs of dorso-intestinal vessels from the internal lacunar network is shown, and marked *l. l.* *h.* is the small vessel by means of which the intestino-tegumentary system of one segment communicates with that of another in the intestinal wall, the small cut branches shown connected with this pass to some portion of the peripheral capillary networks.

FIG. 11.—View of the posterior extremities of the dorsal and ventral vessels.

FIG. 12.—View of a portion of the dorsal vessel cut open along its median dorsal line. *Sept.* Septa. *b. b.* Valves in the septal regions; the anterior one shown entire, the posterior one partially cut away. *D. T.* Apertures of the dorso-tegumentary vessels devoid of any valves. *D. I.<sup>1</sup>*, *D. I.<sup>2</sup>* Apertures of the anterior and posterior dorso-intestinal vessels surrounded by the circular valves. *B.* Transverse section through the same piece of vessel in the region of the apertures of a pair of dorso-intestinal vessels to show the valves *b'.* *W.* The wall of the vessel.

FIG. 13.—External view of the region of the male pores from a spirit-preserved specimen to show the median pit. *gl.<sup>1</sup>* and *gl.<sup>2</sup>* Apertures of the accessory glands. ♂. Male pores.



## On a Little-known Sense-organ in Salpa.

By

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With Plate X.

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THIS organ was mentioned and figured in 1876 by Ussow, in the Memoirs of the Imperial Society of Naturalists of Moscow (T. XVIII, Bd. iii, 2, Moscow, 1876). Since then it has not been mentioned by any author, so far as I have been able to discover; none of the text-books, at any rate, make any allusion to it. Ussow's figure (pl. vii, fig. 49, of the memoir quoted) is very imperfect, missing all of what appear to me to be the most interesting points of the subject. His text is inaccessible to me, being in Russian. I shall do well, I think, to describe the organ as I find it, with only such further reference to the work of the eminent Russian naturalist as may appear useful.

There are, in *Salpa mucronata*, two of these organs. They are end-organs of a recurrent twig of the third nerve. They are symmetrically placed, one on either side of the Salp, on the median frontal plane, at a point about halfway between the large muscle that surrounds the anterior orifice of the mantle, and the most anterior of the three muscle-bands that form the well-known cruciform figure on the central region of the animal. Or, in other words, they are situated on the sides of the Salp, on a transverse plane passing somewhat anteriorly

to the ciliated fossa. These points are shown in fig. 1 (*s. o.*, *s. o.*, the two sense-organs).

By patient manipulation a living *Salpa* may be so placed that an immersion lens may be brought to bear on one of the sense-organs, and the points shown in fig. 2 may then, with some care, be made out. The organ consists of a stem terminating in a bulb, which is surmounted by a delicate hyaline claviform appendage. The stem is seen to be a cellular tube formed by a process of the inner mantle. (Ussow's figure, on the contrary, shows clearly that the author considered that the inner tunica did not penetrate into the tube.) This tube traverses the entire thickness of the hyaline outer mantle or tunica, and swells at its distal end into the bulb. The bulb seems, in favorable specimens, to lie simply in a cup-shaped depression of the tunica. The lumen of the tube, in which the nerve is frequently very clearly shown, is seen to be continued for some distance into the bulb, and to expand there into a cavity or vestibule (frequently somewhat more voluminous relatively to the lumen of the tube than that shown in the figure), in which the nerve breaks up into a delicate arborescence whose twigs terminate by means of small conical swellings on the roof of the cavity (*v.* in the figure). From the distal surface of the bulb delicate tapering hairs are seen to arise by means of a conical base, and to be continued for some distance into the claviform appendage. In the most favorable cases they may be followed for about one third of the length of this appendage, never further in the living state (unless the specimens have been stained *intra vitam* with gentian violet or the like). The appendage itself presents but a single contour, is absolutely hyaline, and gives the impression of being an extremely thin-walled tube or vesicle. This is the view apparently expressed in Ussow's figure, but we shall find that it does not answer to the reality. With great care this single contour may be followed to the bottom of the cup-shaped depression of the tunica spoken of above, but is seen to be continuous there with the wall of this cup; so that the appendage is seen to be a papilla of the hyaline



tunica, and the bulb is seen not to lie strictly speaking in a hollow of the tunica (for it nowhere breaks through the surface of the tunica), but correctly speaking to be surrounded by a deep and narrow annular depression or moat excavated in the substance of the tunica around the bulb, but at a little distance from it (*an.* in the figure). The moat is not figured by Ussow. Little more can be made out by study of the living object.

It may be added here that the shape of the appendage is somewhat variable. It is club-shaped, inasmuch as it is always somewhat thicker at the tip than lower down; but it may swell out very gradually towards the tip, as in fig. 2, or suddenly, as in fig. 3. These two figures represent extreme cases of the relative degrees of swelling that are found. The axis is normally straight, not curved as in Ussow's figure, which I take to represent a pathological state.

In fixed and stained preparations the appearances are very different. As to the stem, it will be sufficient to note that the lining epithelium of the tube is composed of cells similar to those of the inner tunica, of which it is a process—clear cells with somewhat loose-textured nuclei—nuclei in which the chromatic “mitomè” is readily discernible as such.

The bulb, in good preparations, may be analysed into two constituent elements—a centrally placed tuft of sense-cells, and a surrounding cup-shaped wall or calyx of supporting cells. These two groups of cells are readily distinguished by their different optical aspect.

The cells of the calyx are clear cells, in the sense in which that appellation was just now given to the cells of the stem; whilst the sense-cells are dense, stain more deeply, and have compact nuclei in which a normal chromatic mitome can only be made out by careful examination with the very highest powers. Fig. 3 shows these points in a sufficiently realistic manner.

The structure of the calyx is the most difficult point to elucidate in the whole of the organ. In shape the calyx is variable. In Salps of not more than 3 mm. in length it is generally regu-

larly ovoid, or even almost globular (fig. 3). In Salps of from 5 to 7 mm. in length it is more elongated, and presents a contour of an elegant double curvature, the basal portion containing the vestibule being much more drawn out than in earlier stages (see fig. 4). It is composed of clear, flattened, squamous cells, thicker at their proximal margins than at their distal margins. Their nuclei, loose-textured like those of the epithelium of the stem, occupy the proximal region of the cells.

There are two layers of these cells (*ext. ca.* and *int. ca.* in figs. 3 and 4). By reference to fig. 3 it will be seen that doubt is permissible as to the proper way of describing the position of these two layers. The cells marked *int. ca.* appear to be internal with regard to the cells marked *ext. ca.*; but they might also fittingly be described as "upper" or "distal" cells. And seeing that the calyx is evidently only an expanded portion of the tube of the stem, which is lined by only a single layer of epithelium cells, this may appear the more fitting view. I incline, however, to the former view. The impression left on my mind by a most careful study of this object is that the present arrangement is the result of the invagination of the tip of a primitive hollow outgrowth of the inner tunica, the invaginated cells forming an inner layer to the cup thus produced, and the sense-cells being differentiated from the cells at the fundus of the cup, or, if previously formed at the tip of the outgrowth, being invaginated with the rest. It should be added that I have many times distinctly observed the nuclei of the outer cells to be placed on their outer wall, whilst the nuclei of the inner cells were placed on their inner wall, which is the place they would occupy if the latter are only an invaginated state of the former.

Attempts to elucidate this point by direct observation of the genesis of the organ have failed, as it was found that, owing to the very precocious development of the organ, an arduous study of serial sections of the developing stolon would be necessary, and the case does not seem to warrant the expenditure of so much labour.

At the base of the calyx its walls are relatively thick. Towards its mouth they thin down, and terminate in a beautifully delicate, thin, hyaline, incurved lip (*l. ca.* in figs. 3 and 4). According to my view, both layers of cells must concur in the formation of this lip, but are there indistinguishably fused together.

Ussow does not seem to have seen the calyx at all.

The nerve runs down the greater part of the length of the stem without dividing. At or a little before its entrance into the vestibule it begins to break up, first into about three branches, then into about six or seven, and finally, by what appears to me a strictly dichotomous mode of division, into as many twigs as there are sense-cells. This last division always takes place quite close to the sense-cells.

The sense-cells are spindles of about  $15\mu$  in length and  $5\mu$  in breadth. They have an oval nucleus, of very compact texture, somewhat basally placed, with its longer axis parallel to the longer axis of the bulb. They, or at least the more peripherally placed ones, are curved spindles, their outlines following in the main the lines of the calyx, and their distal ends especially being sharply bent inwards, so as to bring them within the orifice of the calyx (which is frequently much narrower than in the figures). I believe that their number is constant, and that there are just fourteen of them in all. They can be counted with considerable certainty in optical transverse sections (see fig. 5).

The sense-cells are embedded in a finely granular matter, which may often be seen to project in a flattened dome out of the mouth of the calyx (*gr.* in figs. 3 and 4). I would suggest that this granular matter is perhaps secreted by the inner cells of the calyx. It is not figured by Ussow.

Proximally these cells taper down gradually into their respective nerve-twigs. Distally they taper down equally gradually, and without the intervention of any "granule" or other apparatus of the sort, into delicate hairs. These hairs are very pale, almost invisible in the living state, smooth or very delicately mottled and corrugated. They stain with great



readiness with certain stains, namely, methyl green, dahlia, gentian, methylen blue, and hæmatoxylin. The stain obtained by the first three of these reagents is not of the normal colour; it is a reddish purple. It takes effect with the greatest energy on the distal portions of the hairs, the basal portions being hardly affected by it (except in the case of an over-stain with hæmatoxylin). The hairs are from  $0.5\mu$  to  $1\mu$  thick, and from  $120\mu$  to  $150\mu$  long.

The aspect of the claviform appendage is totally changed in stained preparations. The fine outer contour described as visible in living specimens is in many examination media quite invisible. If visible in balsam or damar preparations it is invariably greatly shrunken, generally to a far greater extent than is shown in fig. 3. On the other hand, such preparations reveal the presence of a lumen much narrower than could be suspected from the examination of living specimens. It is of approximately equal diameter throughout the length of the appendage, measuring about  $4\mu$  in general; so that the walls of the appendage, instead of being almost immeasurably thin, have a thickness, in the living state, of  $4\mu$  to  $5\mu$  or more. In very shrunken specimens they are practically indemonstrable, and the whole appendage appears reduced to the state of a mere whip, consisting of the sense-hairs and the boundary of the lumen.

The lumen does not pierce the wall of the appendage at its tip; there is no trace of any terminal pore.

The sense-hairs are inserted into the wall of the appendage at the extremity of the lumen. They generally merge insensibly into the substance of the wall, though a slight cone or swelling may sometimes be detected at the point of insertion (*t. s. h.* in fig. 3).

Ussow does not seem to have seen the true lumen, and seems to conceive of the sense-hairs as floating freely in a thin-walled vesicle represented by the outer contour of the appendage.

I have not been able to discover the organ either in the solitary form—*S. democratica*, or in any other *Salpa*.

In the explanation of his figure Ussow calls the organ



a tactile organ. I am altogether disinclined to admit this interpretation. The organ seems to me to have nothing specifically tactile about it. The claviform appendage is wanting in the most essential quality of a mediator of tactile impressions—stiffness. There is nothing hard about it, it is eminently soft and yielding. Compare it with the hard chitinated tactile hair of an Arthropod, say the larva of a Dipteron, and this difference of mechanical principle becomes conspicuous at once. In the Arthropod a stiff appendage, often arranged as a lever—here, the softest of papillæ, a mere jelly!

Looking to the mere essential morphology of the organ, the nature and arrangement of its constituent cells, a different point of view suggests itself. Fusiform sense-cells, with terminal hairs, surrounded by supporting cells, and sunk in a depression of the ectoderm, that is the schema of a taste-bulb. And if the organ of *Salpa mucronata* were not surmounted by the claviform appendage, or if even this appendage were pierced by the smallest pore at its extremity, I suppose no one would be inclined to object to its being considered a taste-bulb. But the presence of the closed appendage does not seem an insuperable objection to this view. The walls of the appendage, though, as I have shown, they are relatively thick, are after all thin structures enough, and that they must be highly permeable is shown by the readiness, above noted, with which the terminal hairs stain with certain reagents. This reaction can be readily obtained in the living state by means of gentian violet or dahlia dissolved in sea water. The penetration is by no means instantaneous, by no means rapid enough to make the organ useful in the selection or rejection of sapid food-substances (which of course, from its position, cannot be its function), but it is perhaps rapid enough to allow it to be useful as an indicator of the chemical quality of the water in which the animal swims.

These considerations appear to me to give much plausibility to the view that the organ is either a taste-bulb, or at least was one once. But there is yet another possible function for which there is perhaps even more to be said—a mode of

action according to which, as it seems to me, this mechanism cannot help acting. The cellulose or "tunicin" mantle of *Salpa* is a fairly tough but highly watery and highly hygro-metric jelly. Given a papilla such as this claviform appendage, with sensory hairs inserted into its tip, if the density of the ambient water be changed, the shape and dimensions of the papilla must be changed in consequence, it must elongate or shorten, and the hairs must be stretched or slackened. If the animal pass into less dense sea water, that is, a medium richer in pure  $H_2O$ , water will be taken up, the appendage will swell and elongate, and the hairs will be pulled. If the animal be carried into water richer in salts the claviform appendage will shrink, and the hairs must be relaxed.

And it seems to me that I have evidence that these actions do actually take place. I have noted above that the shape of the appendage is variable. I have to add here that its surface is typically smooth, but very frequently corrugated or wrinkled into lines of shrinkage. This is especially the case with specimens that have been kept for some time in captivity, and with such as have been treated with chloral hydrate or solutions of intra-vitam staining agents. Even a little methylen blue added to the sea water, in so small a proportion as not perceptibly to stain the hairs, may produce this effect. And in such specimens the hairs themselves, which in perfectly fresh specimens have a smooth and regular contour, are seen to have a markedly corrugated outline, and sometimes a very great degree of curl or twist near the tip.

And, all things considered, I incline to regard the organ as a sensory areometer or hydrometric apparatus.

## EXPLANATION OF PLATE X,

Illustrating Mr. Arthur Bolles Lee's paper "On a Little-known Sense-organ in Salpa."

FIG. 1.—Anterior half of a *Salpa mucronata*. *tu*. The tunicin mantle. *g*. The ganglion. *ci*. The ciliated fossa. *mo*. "Mouth" of the mantle. *s. o.*, *s. o.* The two sense-organs.  $\times 20$ .

FIG. 2.—A sense-organ in the living state. *cl*. The outer contour of the claviform appendage. *an*. The annular depression. *v*. The vestibule of the bulb, in which the terminal arborescence of the nerve is well seen. Optical section.  $\times 1100$ .

FIG. 3.—A sense-organ after preparation (solution of Flemming, Henneguy's acetic-acid alum carmine, damar). *cl*. The outer contour of the claviform appendage. *l*. Its lumen. *l. ca*. Lip of the calyx. *int. ca*. Internal cells of the calyx. *ext. ca*. External cells of the calyx. *gr*. Granular mass in which the sense-cells are embedded. *t. s. h*. Termination of sense-hairs. *an*. Annular depression of the tunicin mantle. *v*. Vestibule of the bulb. Optical section.  $\times 1000$ .

FIG. 4.—A similar preparation without the claviform appendage. The letters as before. Optical section.  $\times 1600$ .

FIG. 5.—A similar preparation seen in optical transverse section. *ca*. The cells of the calyx. *s. c*. The sense-cells, the letters pointing to a fourteenth cell, which is indistinctly seen.  $\times 1100$ .





## Immunity against Microbes.

By

**M. Armand Ruffer, M.A., M.D.(Oxon.)**

### PART I.—A.

WHEN examining the microscopic world actively growing in various infusions, we are often able, says Metschnikoff,<sup>1</sup> “to follow with our eyes the struggle taking place between the representatives of the Flora and Fauna of microbes. Many unicellular animals, such as Amœbæ and other Rhizopoda, as well as flagellated and ciliated Infusoria, feed on various bacteria, devour them in great numbers, and take them into their protoplasm in order to extract from them the necessary nutritive material. Small Monads in the course of a few minutes take into their interior *Leptothrix* filaments ten times longer than they are themselves.” It is a very old observation that Amœbæ and other Protozoa seize dead particles, and, after extracting the nutritive material contained in these, reject the remainder.

According to Metschnikoff,<sup>2</sup> there is no proof that the cells forming the ectoderm of some lower animals—sponges for instance—have the power of taking solid substances into their interior. The same observer, however, has shown that the ectodermic cells of other Metazoa have that power. If, for instance, a small quantity of powdered carmine or indigo be added to water containing *Plumularia* (*Plumularia setacea* or others), some of the carmine or indigo finds its way into the ectoderm of the *Nematocalyces*. These organs send out

<sup>1</sup> ‘Ann. de l’Institut Pasteur,’ 1887, p. 321.

<sup>2</sup> ‘Arbeiten aus dem Zool. Institut in Wien u. Triest,’ vol. v, 1884.

pseudopodia of various shapes, which attach themselves to a calyx or to its contained polyp, or more frequently flatten themselves out around the stem so as almost to encircle it. The ectodermic cells of the free extremities fuse into a common protoplasmic mass, which sends out some few pseudopodia. The slow creeping movements of the ends of the Nematocalyces serve to clean the neighbouring polyps—a function accounting for the frequent presence of foreign particles in their ectoderm. The Nematocalyces of *Plumularia*, moreover, eat up three parts of the animal which has died—the polyp heads, for instance—and are frequently crammed with dead particles which they have ingested. Nematocalyces, therefore, have a double function; namely, that of scavenging the outside of the animal by creeping along it, and that of eating up useless tissues. The ectodermic cells of the larvæ of *Setinæ* also absorb solid material after enclosing it with their pseudopodia.

Intracellular digestion by ectodermic cells, however, is not observed frequently, whereas the absorption and digestion of solid particles by mesodermic structures are easily demonstrated. The nutrition of fresh-water sponges, for instance, is carried out by wandering cells, which correspond to the mesodermic cells of higher animals.<sup>1</sup>

Häekel<sup>2</sup> noticed the fact that particles of indigo when injected into one of the Mollusca (*Thetys*) are absorbed by the corpuscles of the blood, but, although this contribution to the knowledge of the functions of mesodermic cells is important, still more may be learnt from the study of processes taking place during the destruction of useless organs in the period of metamorphosis of Invertebrata.

Taking *Auricularia*<sup>3</sup> as an example, we find that resorption-phenomena occur during two stages of its life history, namely, during the assumption of the pupa-stage, when a part of the longitudinal ring of cilia is lost—that is, is disintegrated and

<sup>1</sup> Compare, Metschnikoff, 'Zeitschr. f. wiss. Zool.,' vol. xxiv, p. 10, and T. E. Schulze, *ibidem*, vol. xxv, p. 258.

<sup>2</sup> Radiolarien, 1862, p. 104.

<sup>3</sup> Metschnikoff, 'Quart. Journ. of Micr. Sci.,' vol. xxiv, p. 10.

devoured by the mesoderm—and, secondly, during the metamorphosis of the pupa into a young *Synepta*, when mesodermic cells become active again, collect beneath the ciliated rings, and eat up the products of disintegration. Every amœboid cell during both these stages is generally loaded with an enormous mass of débris-granules contained in the interior of clear vacuoles.

In *Asterides* also, whole segments of larvæ are absorbed by mesodermic cells during the metamorphosis. This process, in fact, appears to be the rule in all Echinoderms.

Mesodermic cells were supposed by Ganin<sup>1</sup> to play an active part in the histolysis of muscular fibres during the metamorphosis of flies. More lately, Kowalewsky<sup>2</sup> showed that Ganin's supposition was correct, for he proved that large numbers of wandering cells penetrate between the muscular fibres, take into their interior and digest the latter.

Mesodermic cells are active not only during the period of metamorphosis, but under many other circumstances. Schneider<sup>3</sup> noticed that the resorption of the genital organs of *Hirudo* is carried out by amœboid cells greatly resembling blood-corpuscles, and Metschnikoff described the same phenomenon as taking place in the *Aurelia aurita*.<sup>4</sup> The developing nemertine in a pilidium also, if the latter is left for some length of time in a glass vessel, atrophies and is devoured by amœboid mesodermic cells. Some of the mesodermic cells of transparent salt-water animals taken fresh from the sea are filled with foreign matter, and Metschnikoff assumes that such foreign bodies gain an entrance by piercing through the body-wall, being ultimately swallowed by amœboid cells. Particles of carmine suspended in water not only penetrate into the cells of the entoderm, but into those of the mesoderm also.

The following experiments throw much light on the process of intra-cellular digestion by mesodermic cells. When

<sup>1</sup> Ganin, 'Beiträge, &c.,' 1876, p. 40.

<sup>2</sup> Kowalewsky, 'Zeitschr. f. wiss. Zool.,' vol. xlv, 1887.

<sup>3</sup> Schneider, 'Zool. Anzeiger,' 1880, p. 19.

<sup>4</sup> Metschnikoff, loc. cit., p. 89.

carmine or indigo powder suspended in water is injected under the ectoderm of *Bipinnaria asterigera* or *Phyllirrhoe bucephalum*, the coloured powder is soon taken into the interior of the mesodermic cells. In *Bipinnaria* the carmine is absorbed by both large and small cells; in *Phyllirrhoe*, on the other hand, the powder is ingested by the small cells, the larger ones containing dissolved carmine only. The small particles of carmine are actually in the interior of cells, but the larger particles of the same matter become surrounded by numerous small cells which form plasmodia resembling the giant-cells found in the pathological structures of vertebrata. Similar plasmodia formed by mesodermic cells are formed when a few drops of human blood are introduced under the cuticle of *Bipinnaria asterigera*; the nuclei of such plasmodia lie at the periphery, whilst the centre of the plasmodium contains a mass of red blood-corpuscles in different stages of degeneration.

Migrating cells also form a barrier around foreign bodies, such as glass, cellulose, &c., which are introduced under the skin of these animals; but, in many cases, no plasmodia are seen, so that the formation of the latter structures does not necessarily follow on the introduction of foreign bodies into these animals.

Blood-vessels, if present, do not necessarily play a part in the process of inflammation, for no transudation takes place from them, provided the foreign body be introduced without wounding a blood-vessel. Cohnheim's dictum, therefore—"Ohne Gefäße keine Entzündung"—is not altogether correct, or, as Metschnikoff brilliantly says, "Inflammation is, genealogically speaking, of a much older date than the formation of vessels, and exudation is a comparatively late phenomenon."

Mesodermic cells, however, do not eat everything they come across, but have the power of exerting a choice. Thus the mesodermic cells of *Phyllirrhoe* make no attempt to destroy the fresh eggs of *Sphærechinus granularis* injected under the skin of the animal. Nay, more; the eggs retain their vitality, so that they may be fecundated artificially whilst



living in the animal's tissues, and may form normal blastulæ. Contrariwise, the living spermatozoa of *Sphærechinus granularis* introduced under the skin of *Phyllirrhoe* soon fall a prey to mesodermic cells.

The mesodermic cells of *Bipinnaria asterigera* also eat and destroy the necrosed parts of the animal.

The function of amœboid cells<sup>1</sup> is not limited to the absorption of weakened or dead tissues; but the same structures take an active part in the fight between the animal organism and the surrounding microbes. When a solution crowded with micro-organisms is injected under the skin of *Bipinnaria* or *Phyllirrhoe*, the parasites, whether mobile or not, are soon taken into the interior of the protoplasm and vacuoles of mesodermic cells, and gradually digested by the latter. The tunica of *Botryllus*, for instance, even in an animal taken fresh from the sea, always contains colonies of micro-organisms. The latter are actively pursued by the wandering cells of the tunica, which digest them after swallowing them up. The struggle is not a one-sided one, however, for the victory often remains with the micro-organisms, as is proved by the presence of dead amœboid cells containing a number of dead bacterial filaments radiating out of them.

Similar facts have been observed in *Daphnia* by Metschnikoff.<sup>2</sup> These small fresh-water crustaceans are frequently invaded by a fungus belonging to the yeast family (*Monospora bicuspidata*). The long needle-like spores of the parasite penetrate with the food into the alimentary canal, pass into the body-cavity after perforating the walls of the intestine, quickly invade the entire body, and often kill the animal. Whilst the disease is in active progress the leucocytes still strive to fight, and absorb numbers of conidia. The latter, however, rapidly multiply, and destroy the amœboid cells, the victory ultimately falling to the parasites.

The study of the development and of the life-history of Vertebrata affords many examples showing that the meso-

<sup>1</sup> 'Annal. de l'Institut Pasteur,' 1887, p. 323.

<sup>2</sup> Ibid., p. 325.

dermic structures of higher animals have similar functions. A few instances will suffice to illustrate this.

When the tadpole passes into the frog stage, the muscles and nerves of the tail gradually disappear as they become a prey to amœboid cells, which surround them and eat up the tissue which still presents the structure of normal muscular fibres. This process is precisely similar to that which Kowalevsky has observed during the metamorphosis of flies.

Again, amœboid mesodermic cells collect round the muscles of the tail of living, uninjured *Bombinator* larvæ<sup>1</sup> at the beginning of the metamorphosis, and gradually devour these structures. During the progress of the atrophy of the gills also, the presence of large fully-laden mesodermic cells may be easily demonstrated.

The mesodermic cells of higher Vertebrata have similar functions. The resorption of osseous substance<sup>2</sup> constantly taking place in the shaft of long bones is essentially dependent on the presence of large multinucleated cells (Osteoclasts, Myeloplaxes of Robin), which excavate small shallow pits (foveolæ) in the part which is undergoing absorption.

Large mono-nucleated cells containing red blood-corpuscles in their interior are also met with in the spleen.<sup>3</sup> These intracellular red blood-corpuscles may be normal in appearance, but more frequently they appear to be disintegrated and digested in the interior of these large spleen-cells, a few pigment-granules representing all that remains of them. Many observers have succeeded in watching the process of intracellular digestion of red blood-corpuscles actually taking place.

Mesodermic cells not only devour other structures, but prey on one another also. According to Heidenhein, the walls of the intestinal canal of certain animals,<sup>4</sup> more especially guinea-pigs, are lined by epithelium cells, between which large mesodermic cells find their way to the surface and absorb

<sup>1</sup> Metschnikoff, 'Quart. Journ. of Micr. Sci.,' vol. xxiv, p. 112.

<sup>2</sup> Kölliker, 'Die normale usorption des Knochengewebes,' Leipzig, 1873.

<sup>3</sup> Bardach, 'Ann. de l'Institut Pasteur,' Dec., 1889, p. 599.

<sup>4</sup> Heidenhein, 'Pflüger's Archiv,' vol. xlvii, 1888.

smaller lymphocytes, which are ultimately digested in the interior of the larger cells.

The writer<sup>1</sup> found in the lymphoid structures (tonsils, Peyer's patches, mesenteric glands) of the alimentary canal of many animals large wandering cells containing two, three, or more lymphocytes in their interior, and described all the stages of the intracellular digestion of the latter.

More lately, the writer<sup>2</sup> has discovered that the so-called epithelioid cells of the spleen-pulp have the power of taking into their interior and of digesting smaller amœboid cells. The same cells also swallow inert substances such as vermilion when this is injected into the blood-stream, this fact showing that they are amœboid structures—a supposition suggested by their irregular shape.

Before proceeding further, it is necessary to discuss the origin of these cells—Phagocytes, as Metschnikoff has called them. "The function of Phagocytes," says Metschnikoff,<sup>3</sup> is usually the property of two kinds of cells. Small cells (migrating cells) possessing one or many nuclei—leucocytes in the narrower sense of the term—are scattered through all tissues and concentrated in the lymphatic and blood-system, but emigrate in case of need to any part of the body which is invaded by parasites. I give to these cells the comprehensive name of Microphages. On the other hand, I give the name of Macrophages to the fixed cells of connective tissue, the epithelioid cells of pulmonary alveoli, in fact, to all kinds of structure which possess the power of taking solid bodies into their interior, and which are provided with a single large nucleus, less easy to stain than the nuclei of Microphages." This definition, although clear, is, as will be seen later on, not quite adequate.

Since Metschnikoff's first paper appeared it has become evident that the Macrophages are often, if not always, derived from Microphages. Heidenhein,<sup>4</sup> describing the Macrophages

<sup>1</sup> Armand Ruffer, 'Quart Journ. of Micr. Sci.,' February, 1890.

<sup>2</sup> The writer gave a short account of these researches before the British Medical Association, August, 1890.

<sup>3</sup> Metschnikoff, 'Ann. de l'Institut Pasteur,' 1887, p. 324.

<sup>4</sup> Heidenhein, loc. cit.

found in the intestinal canal, says, "Now there does not appear a doubt that these large giant-cells are developed from the ordinary lymphocytes," and proceeds to give figures showing their gradual development. In the writer's paper on the "Phagocytes of the Alimentary Canal,"<sup>1</sup> he showed that the Macrophages of the Peyer's patches and tonsils are derived from small lymphocytes, and he described the mode of development of these enormous cells. In the spleen-pulp and venous sinuses of the same organ also the writer has been able to trace the development of the Macrophages from Microphages, for all intermediate stages between them can easily be observed.

It has been shown that the mesodermic cells of invertebrata not unfrequently come to the surface, scavenge the outside of the animal's body, and remove any dead particles which they may come across. In vertebrata the same process takes place in the mucous membranes lining the cavities of the body which are in contact with the external world. Eberth<sup>2</sup>, as early as 1864, noticed that wandering cells force their way between the epithelium cells covering the intestinal canal. Ph. Stöhr<sup>3</sup> then drew general attention to the fact that this emigration takes place along the whole length of the alimentary tract. The writer has shown that the leucocytes of dogs wander to the free surface of the tonsils, take particles of dust, charcoal, &c., into their interior, and carry them into the depths of these organs. Here the Microphages and their contents frequently fall a prey to the Macrophages present in these structures. This fact proves that the mesoblastic cells of vertebrata have functions similar to those of invertebrata, but evidence far more conclusive can be obtained by observing what takes place in the lungs of various animals.

Particles of charcoal, dust, &c., are to be met with in the epithelioid cells (Saub zellen) of the lungs of most animals. The precise nature of these cells remained uncertain until the

<sup>1</sup> Armand Ruffer, loc. cit.

<sup>2</sup> Eberth, 'Hürzburger Naturwissenschaft. Zeitschrift,' 1864, p. 23.

<sup>3</sup> Ph. Stöhr, see Heidenhein, loc. cit.



experiments of Tschistowitsch<sup>1</sup> demonstrated the fact that they belong to the group of Phagocytes, and are therefore probably derived from ordinary lymphocytes. These cells remove particles of foreign matter which have entered the air-passages, and carry them to the neighbouring lymphatic glands, where they are again arrested and often remain permanently. In the bronchial glands of London cats, for instance, which always contain a large quantity of charcoal, the particles of charcoal, dust, &c., are contained in the interior of large mono-nucleated epithelioid cells which are real Macrophages.

The free surface of vertebrata, like the free surface of invertebrata, is everywhere in contact with a large number of micro-organisms. Nevertheless, the internal organs of the body, the liver, kidneys, spleen, &c., contain no parasites, and it follows that there must be some mechanism preventing the entrance of micro-organisms into a healthy animal's tissues, or some means of destroying them should they gain admittance.

The skin of all animals is an impenetrable barrier to the entrance of micro-organisms, and, to all appearances, the latter do not make an attempt to penetrate through the same into the tissues. It is plain that micro-organisms cannot easily force their way through several layers of hard epithelium cells, such as go to form the outer skin of most mammals or cover the surface of the lips and tongue; but it is evident also that micro-organisms cannot penetrate through a single layer of epithelium cells. In sections passing through the walls and contents of the intestines of any animal, an appalling number of microbes are seen lining the free border of the layer of epithelium cells, but none are found in or between the latter. The epithelium cells therefore, together with the cementing substance between them, form a satisfactory barrier to the entrance of microbes.

Matters, however, are not quite so simple as they appear, for in some organs, of healthy animals even, a struggle takes place during every hour of the day between micro-organisms and mesodermic cells.

<sup>1</sup> Tschistowitsch, 'Ann. de l'Institut Pasteur,' July, 1889.

In 1887, Metschnikoff found that the leucocytes present in the mucus covering the tonsils of healthy people frequently contained micro-organisms. In 1885, Ribbert and Bizozzero examining the Peyer's patches and vermiform appendix of healthy rabbits, showed that the walls of these organs are crammed with an enormous number of degenerated micro-organisms; both these observers attributing the death of the parasites to the action of the cells of the part. In 1890, the writer<sup>1</sup> demonstrated the fact that the leucocytes which wander out between the epithelium cells to the surface of the lymphoid organs of various animals—more especially rabbits—are frequently filled with micro-organisms. The leucocytes (microphages) carry their prey into the interior of the tissues, but, being weakened by the secretions of the microbes they contain, are frequently eaten up by larger cells (macrophages), which resemble in all particulars the macrophages described by Metschnikoff in pathological specimens. The writer has shown that the same process takes place in the tonsils also. The conclusion to be drawn from these researches is, that the mesodermic cells, of healthy animals even, have the power of absorbing, destroying and digesting micro-organisms.

A little consideration shows that the lungs and intestinal tract, which are the organs most likely to be attacked by micro-organisms, are provided with a large number of mesodermic cells. Attention has been drawn to the fact that mesodermic cells proceed to the surface along the whole length of the alimentary canal and destroy micro-organisms. Moreover, immediately below the layer of epithelium cells and along the whole length of the intestinal tract, a layer of adenoid tissue is spread out like a lymphatic gland. Every micro-organism, therefore, which, by any chance, passes the first barrier of epithelium cells must run the gauntlet of this adenoid layer and most probably be destroyed.

In the lungs, similar macrophages line the alveoli and remove dust, particles of charcoal, &c., which, by gradual accumulation, might choke the air-passages. Surgeons know—and Sir

<sup>1</sup> Armand Ruffer, *loc. cit.*

Joseph Lister was the first to draw attention to that fact—that, should the skin remain intact, a wound of the lung rarely becomes septic. Moreover, the elegant researches of Tyndal, Gunning, Straus and H. Dubreul, and Straus<sup>1</sup>, prove that expired air contains no microbes—that is, has been filtered in the lungs. Other investigations have demonstrated the fact that the germs stick to the moist surfaces of the upper air-passages, and that very few only are carried into the lungs. Doubtless some are carried into the alveoli, just as carbon-particles are carried there.

M. Tschistowitsch<sup>2</sup> has lately shown what becomes of micro-organisms which have been introduced into the lungs. He found that micro-organisms which are non-pathogenic for the animal used, are at once attacked by the phagocytes of the lung which swallow, digest, and destroy them; the safety of the animal being therefore dependant on the efficient action of these cells. It follows that the bacilli which are carried by the stream of air into the lungs, must at once be seized upon and destroyed by these phagocytes. The fact that the lung consists of a number of small aseptic cavities can thus be readily explained.

Having now studied the functions of wandering mesodermic cells in healthy animals, let us see how these same cells react when micro-organisms find their way or are artificially introduced into an animal's tissues.

<sup>1</sup> For the literature on this subject see Straus, 'Ann. de l'Institut Pasteur,' 1888, p. 181.

<sup>2</sup> Tschistowitsch, loc. cit.





On a New Species of Phymosoma, with a Synopsis of the Genus and some Account of its Geographical Distribution.

By

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With Plate XI.

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DURING a visit to the Bahama Islands in 1887, Professor Weldon made an excursion to the neighbouring island of Bimini. Whilst investigating the fauna of the lagoon in that island he came across a few specimens of what appeared to him to be a new species of *Phymosoma*. One or two of these he dissected on the spot; the remaining three he brought to England, and was good enough to give them to me for description at the time when he handed over to me his specimens of *Phymosoma varians* which form the subject of a previous memoir.

I have divided this paper into the following sections:

- (i) A general description of the new species.
- (ii) A detailed description of those organs which differ markedly from the similar organs in *Ph. varians*.
- (iii) A synopsis of the genus.
- (iv) A short account of the geographical distribution of the genus.

## PART I.

Genus *Phymosoma*.*Phymosoma Weldonii*, n. sp.

The length of the body is 3.5 cm. in the largest specimen, 3.25 cm. in the second, and 3 cm. in the smallest. The greatest width is 1 cm. At the base of the introvert, which was in each specimen retracted, the width is 2 mm. The body has a plump appearance and is slightly curved (fig. 1).

The ground colour of the preserved specimens is light buff, which is modified by the presence of dark brown papillæ; these are so numerous as to give the animal as a whole a dark brown colour. The papillæ are of two kinds (figs. 2 and 3).

No hooks or traces of hooks can be detected on the introvert.

The mouth is surrounded by a vascular lower lip, which in the dorsal middle line is continuous with the outer limbs of the lophophore. The latter structure is in the shape of a double horseshoe, the outer semicircle of tentacles corresponding to the lophophore of *Ph. varians*. In the ventral middle line this outer limb is bent dorsalwards, and thus the second horseshoe is produced (fig. 5). The lophophore bears a great number of long tentacles, seventy or eighty (figs. 5 and 8).

Behind the head is a well-developed collar, pigmented on the anterior surface (fig. 5).

The alimentary canal is tightly coiled, the number of twists being twelve or thirteen (fig. 4). It is supported by a well-developed spindle-muscle, which passes up the axis of the coil, and is attached at one end to the longitudinal muscles of the body-wall in the neighbourhood of the anus, and at the other to the posterior end of the body-wall. The anus is situated at the line of junction of the two kinds of papillæ.

The longitudinal muscles are arranged in ten or twelve bundles in the anterior half of the body; in the middle of the body there are about twice that number, as each bundle splits into two. These fuse together again at the posterior end of the body. At the base of the introvert the usual inversion of

layers occurs, the longitudinal muscles fusing into a continuous sheath, the circular muscles becoming broken up into bundles.

The retractor muscles are two in number, a right and a left; they arise about the level of the junction of the anterior two thirds with the posterior third of the body. They embrace the œsophagus, forming a semicircular band of muscle-fibres which are wanting only in the dorsal middle line where the heart lies.

The heart is provided with very numerous cæcal diverticula (figs. 4 and 7).

The external aperture of the kidneys lies on a level a little behind that of the anus.

Habitat: the lagoon, Bimini Island, the Bahamas.

## PART II.

### The Papillæ.

The papillæ of the skin are of two kinds, those on the body and those on the introvert. In the middle of the trunk the papillæ have an oblong outline, and are arranged in very regular rings (figs. 1 and 3); near the posterior end of the body, and also at the base of the introvert, the papillæ are so crowded together as to lose their rectangular outline. These papillæ are of a dark brown colour, and in those regions where they are crowded together the buff colour of the rest of the skin is completely obliterated. The trunk papillæ form only low elevations above the general level of the skin; each has a central pore, surrounded by a number of brown horny plates, which are modifications of the cuticle. These plates show a faintly laminated structure; they are represented in section in figs. 11 and 12.

Between these brown plates are placed a number of deeply pigmented granules of a dark brown, almost black colour. These give the dark brown colour to the papillæ (figs. 9 and 12). Neither the plates nor the pigment granules show any trace of being connected with any special cells; they seem to be modifications of the cuticle. The papillæ, like those of *Ph. varians*, are formed by the ectoderm-cells rising up and invaginating to form a double cup. The outer wall of the cup is formed by

ectodermal cells which have kept their ordinary character, and the inner wall is composed of a few cells of enormous size, which all but obliterate the cavity of the cup. These cells are wedge-shaped, and their broader ends are crowded with small spherical concretions, which do not dissolve in alcohol, chloroform, or benzine. Towards their outer, narrower ends these cells become free from these concretions and stain uniformly, or else they contain from two to five or six large star-shaped aggregations of crystals (fig. 9). Nothing like these were seen in the papillæ of *Ph. varians*.

The papillæ on the introvert stand out much farther from the level of the skin than those of the trunk (fig. 2). They are conical in shape, with a narrow basis. At their apex is a pore, and microscopic sections show that this is surrounded by a number of minute horny plates similar to those in the papillæ of the trunk, though much smaller, and not visible like the latter on the surface view. No crystals were found in the papillæ of the introvert, but the large wedge-shaped cells were in other respects similar to those described in the trunk papillæ.

#### The Head.

The head is represented diagrammatically in fig. 5. Part of the outer limb of the double horseshoe-shaped lophophore, involving eight or ten tentacles, has been cut out in order to show the pigmented region in the hollow of the original lophophore, and to display more clearly the arrangement of the tentacles. The thin transparent collar is extended over the head, a condition in which it is usually found when the introvert is retracted. The crescentiform mouth is shown surrounded by the lower lip, and at the base of the pigmented region on the dorsal side lies the brain, directly continuous at each side with the epidermis.

The tentacles are very numerous, seventy to ninety. Like those of *Phymosoma varians*, they are roughly triangular in section. One side, that directed towards the mouth, is grooved, and the groove is lined with cilia; the grooves of the various tentacles tend to fuse together near their lower ends,



and are directly continuous with the ciliated grooves on the wall of the œsophagus (fig. 8). A nerve runs along the base of the groove, and on each side of the nerve is a blood-vessel; the third blood-vessel occupies the angle opposite the side bearing the groove: all these three vessels anastomose occasionally, and communicate below with a large blood-sinus at their base.

In the diagram the plane of the tentacular crown is too flat; instead of being at right angles to the long axis of the body it should be raised up, and in a manner overhanging the mouth: in other respects the figure represents the disposition of the parts, although rather diagrammatically.

The sides of the tentacles which are directed away from the mouth are deeply pigmented, and the pigment is continued into a hollow at their base (figs. 5 and 8). This hollow lies partly between the two horseshoes of the tentacles, and is therefore itself horseshoe-shaped; at its dorsal end the depression becomes deeper, and lodges the brain.

The most important difference between the tentacles of *Ph. Weldonii* and *Ph. varians* lies in the absence of the rows of those skeletal cells which formed so interesting a feature of the latter species. Their place is occupied by a well-developed fibrous connective tissue, which passes down into the base of the lophophore, and is then continuous with the connective tissue which surrounds the œsophagus, and which serves as a point of attachment to the retractor muscles. The lower lip is also devoid of any skeletal structures; it is, however, very vascular; its inner surface is ciliated, the cilia being continued down the œsophagus; its outer surface is pigmented.

The area between the lower lip and the collar, as well as the inner surface of the latter, is also pigmented, although it has not been possible to represent this in the diagram. This continuous lining of pigment ceases at the edge of the collar; its outer surface is not pigmented. The collar is represented in fig. 5 completely expanded, and covering in the head; it is usually found in this condition when the introvert is retracted. It would be interesting to know whether it is ever expanded in this way when the head is extended during life. I have

never seen it in this condition in those specimens of the unarmed *Gephyrea* which I have been able to examine alive.

The absence of hooks on the introvert is a marked feature of this species of *Phymosoma*. Only five other species of the genus are devoid of these characteristic chitinous structures.

### The Vascular System.

The vascular system of the unarmed *Gephyrea* consists of a closed space which has no capillaries in connection with it. The system is distributed in the various parts of the head, and its chief function would seem to be that of distending the tentacles and lower lip. In the tentacular blood-vessels the blood is separated from the surrounding water by a thin layer of tissue, and it is very probable that it becomes aërated during its passage through these organs. Its function as a carrier of oxygen cannot be of very great importance, since the system is entirely confined to one small part of the body. Probably those organs outside the head are dependent for their aëration on the corpuscles in the perivisceral fluid, though it is not easy to see where these can get their supply of oxygen and eliminate their waste matter.

The large vessel which lies on the dorsal surface of the œsophagus, and which is usually known as the heart, acts as a reservoir into which the blood retires when the tentacles are retracted (figs. 4 and 7). In *Ph. varians*, where there were few tentacles, the heart was a straight blind sac about .5 cm. long, extending along the dorsal side of the œsophagus; but in *Ph. Weldonii* the number of tentacles is much greater, and the reservoir is correspondingly increased. The heart is much longer, being continued along the dorsal side of the œsophagus for a centimetre or two, and thus becoming involved in the twisting of the alimentary canal (fig. 4). Its capacity is also much increased by numerous small diverticula, which project as finger-like processes, and give the heart a very characteristic appearance. The walls of the heart and its diverticula are thin, with few muscle-fibres in its substance. Similar diverticula occur in the species *Ph. antillarum*, *Ph. pelma*, and

*Ph. asser*; and *Ph. nigrescens* has smaller diverticula of the same kind.

The corpuscles contained in this closed system are of two kinds—large clear cells with a well-developed outline, a well-stained nucleus which lies at one side of the cell, and apparently no cell-contents; the other corpuscles are smaller, with a protoplasmic body which stains well, and a nucleus in the centre.

In addition to the fluid of this closed system of spaces, the general fluid of the body-cavity contains corpuscles and the ova or sperm morulae.

### The Brain.

The nervous system has the same arrangement of nerve-fibres and ganglion-cells as that which I described in *Ph. varians*. The brain occupies the same relative position, situated at the dorsal side of the lophophore at the base of a slight depression. This depression is much smaller than the similar one in *Ph. varians*; its area being encroached upon by the bending back of the tentacular crown to form the inner horseshoe, the space is thus rendered rather slit-like and slightly curved (figs. 5 and 8). The depression is lined by a curiously crumpled and deeply pigmented epidermis, with which the brain is in direct continuity in two places. The shape of the brain is different from that of the other species, and this difference corresponds with the alteration in shape of the pigmented area in which it lies. It is bilobed, but the grooves between the lobes are very slight. Each lobe of the brain is smaller in transverse section than those of *Ph. varians*; on the other hand, their long axis is much longer, so that each lobe is slimmer and more elongated. The narrow outer end of the lobe bifurcates into two stout nerves, one of which passes round on each side in the connective tissue surrounding the walls of the œsophagus, and fuses with the similar one of the other side to form the ventral nerve-cord. The other passes up into the lophophore in the middle dorsal line, and then turns outwards and runs along the base of the tentacles, giving off a branch to each. There is a second lophophoral pair of nerves of small

size, which run into the outer end of the crown of tentacles where it fuses with the dorsal ends of the lower lip. I could not make out very satisfactorily whether these nerves supply branches to the tentacles of this region, but I am inclined to think that they do.

A pair of very minute nerves leave the brain close to the middle line; these run to the pigmented tissue of the depression at the bottom of which the brain lies. At about the point of the greatest circumference of each lobe the ganglion-cells of the brain are in continuity with this pigmented epithelium, and at one spot this epithelium is involuted into the substance of the brain, its cells become enlarged and crowded with pigment granules of dense black. The lumen of the involution is practically occluded; these pigmented involutions form the eyes.

The ganglion-cells form a cap which wraps round the fibres except for about a quarter of the circumference, where they come to the surface; this fibrous portion is ventral and posterior in position. The whole brain is half surrounded by the large blood-sinus into which the dorsal vessel opens anteriorly, and which gives off the vessels to the tentacles.

The arrangement of the fibres and ganglion-cells in the ventral cord is the same as that of *Ph. varians* (fig. 10).

The remaining organs of *Ph. Weldonii* resemble those of *Ph. varians* so closely as to render any detailed account superfluous. The nephridia are two in number (figs. 4 and 7). The relationship of their external and ciliated internal openings is shown in fig. 11. The outer wall of the nephridium is, as this figure shows, continuous with the body-wall, but this is for a short distance only. The organ soon becomes free, and stretches back to the end of the body, and then in its most extended condition may be bent back again.

In its histological details the structure of the nephridium is similar to that which I described in my former paper. The inner surface is broken up into a series of crypts which are lined by large glandular cells. Outside these is a meshwork of muscle-fibres which I have endeavoured to depict in fig. 6,



and covering these again is the layer of flat peritoneal epithelium.

The very curious mode by which the glandular cells of the nephridium in *Ph. varians* excrete their waste products, by casting off vesicles into the lumen of the organ, is repeated in this species. These vesicles contain granules. Their method of formation and of breaking off from the free end of the secreting cell is paralleled by the secreting cells which line the mammary glands of Mammalia, or by the cells which give rise to the secretion of the liver in *Astacus*.

The generative organs consist of a band of modified peritoneal epithelium which lies at the base of the retractor muscles. The generative products split off into the peritoneal cavity.

### PART III.

#### A Synopsis of the Genus *Phymosoma*.

In the admirable systematic monograph<sup>1</sup> on the Sipunculidæ, in which Prof. Selenka, with the assistance of Dr. de Man and Dr. Bülow, published the results of their work on the Gephyrea collected by Prof. Semper in the Philippine Archipelago, a synoptical table of the genus *Phymosoma*, which included at that time eighteen species, is published. Since 1883, the year of the publication of the above-mentioned work, nine new species have been added to the genus. Eight of these are due to the energy of Dr. Sluiter,<sup>2</sup> who has done so much to increase our knowledge of the marine fauna of the Malay Archipelago. The ninth was found by Prof. Weldon in the Bahamas. It will thus be seen that since the publication of Selenka's synopsis the number of described species of *Phymosoma* has increased by half the original. I have, therefore, prepared the following table, which is largely founded on Selenka's, but which includes those new species of the genus which have been described since the publication of his monograph.

<sup>1</sup> In a note in my previous paper on *Ph. varians* this work was inadvertently attributed to Prof. Spengel.

<sup>2</sup> 'Beiträge zu der Kenntniss der Gephyreën aus dem Malayischen Archipel,' von Dr. C. Ph. Sluiter.

Hooks absent

|                     |                                                                                                                                                                  |   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |   |                               |
|---------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|---|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---|-------------------------------|
| I. Four retractors  | Introvert shorter than body. Small tropical species.                                                                                                             | { | Introvert as long as body. Papillæ conical. Large species. Norway . . . . .<br>Body papillæ formed of many concentric plates, which decrease in size peripherally; at the hinder end they are in contact. Antilles, Chili, Punta Arenas . . . . .<br>Body papillæ of very few concentric plates. Large square plates lie scattered in the skin between the papillæ. Batjan, Billiton . . . . .<br>Body papillæ formed of very few concentric plates. On the body itself the papillæ are far apart, and the skin between them is provided with countless little irregular bodies. Mauritius, Java, Philippines . . . . .<br>Body papillæ elliptical, with well-defined limits. No angular bodies between papillæ. The proboscis has small spines. Java . . . . . | { | 1. Lovénii, Kor and Dan.      |
|                     |                                                                                                                                                                  |   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |   | 2. Antillarum, Gr. and Oerst. |
|                     |                                                                                                                                                                  |   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |   | 3. Asser, Sel. and de Man.    |
|                     |                                                                                                                                                                  |   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |   | 4. Pelma, Sel. and de Man.    |
| II. Two retractors  | Introvert shorter than body . . . . .                                                                                                                            | { | Papillæ in trunk square, not raised; in proboscis raised and conical. Bahamas . . . . .                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | { | 5. Psaron, Sluit.             |
|                     |                                                                                                                                                                  |   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |   | 6. Weldonii, Ship.            |
| I. Two retractors   | Papillæ egg-shaped. 11—15 rows of very small hooks. Skin flesh-coloured to white. Red Sea . . . . .                                                              | { | Papillæ on body white, chiefly on dorsal surface. 38 closed rows of hooks and 25 open. Skin white and transparent. Anus far back. Malay Archipelago . . . . .                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | { | 7. Rüppellii, Grube.          |
|                     |                                                                                                                                                                  |   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |   | 8. Diaphanes, Sluit.          |
| II. Threeretractors | Hooks short, bent, and laterally compressed. Body covered with papillæ                                                                                           | { | Anterior end of body sharply separated from the remainder, and thickly covered with papillæ. Few papillæ on middle of body. A few rows of sickle-shaped hooks. Malay Archipelago . . . . .<br>The whole body covered with pointed conical papillæ. Very numerous rows of hooks. Red Sea . . . . .<br>Retractors not arising at the same level                                                                                                                                                                                                                                                                                                                                                                                                                   | { | 9. Falcidentatum, Sluit.      |
|                     |                                                                                                                                                                  |   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |   | 10. Asperum, Grube.           |
|                     | Retractors arise posteriorly, and after a short course fuse. Circular muscle layer in bundles . . . . .                                                          | { | 60—70 rings of hooks, 17—18 longitudinal muscles. Philippines, Singapore. 22 rings of hooks, 23 longitudinal muscles. Amboyna . . . . .                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | { | 11. Lurco, Sel. and de Man.   |
|                     |                                                                                                                                                                  |   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |   | 12. Maculatum, Sluit.         |
|                     | Retractors of usual length, never arise posteriorly. Hooks with folds at their bases                                                                             | { | The body very rough and hard. Numerous rows of hooks, 80—100. Papillæ crowded. East Indies, Mauritius, Red Sea . . . . .<br>Papillæ irregularly scattered. 40 rows of very minute hooks. Malay Archipelago . . . . .<br>Segmental organs not remarkably long                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | { | 13. Pacificum, Kef.           |
|                     |                                                                                                                                                                  |   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |   | 14. Microdontoton, Sluit.     |
|                     | Hooks with a multituberculate secondary tooth. Brown tube with a saccular diverticulum on its inner mouth. Large species, with long introvert. Panama, Mauritius | { |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | { | 15. Pectinatum, Kef.          |

III. Four retractors

|                                                                                                                                                                                 |                                  |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|
| Hooks without secondary processes. No diverticulum on brown tubes                                                                                                               |                                  |
| A row of large spines on dorsal surface of posterior half of introvert. Philippines<br>No such spines on introvert                                                              | 16. Dentigerum, Sel. and de Man. |
|                                                                                                                                                                                 | 17. Nigritorquatum, Sluit.       |
| Only 4 rows of hooks. An intensely black row of granules and tubercles round mouth. 20—22 longitudinal muscles. Batavia<br>More than 4 rows of hooks                            |                                  |
| Hooks bent through a right angle.                                                                                                                                               | 18. Varians, Kef.                |
|                                                                                                                                                                                 | 19. Albolineatum, Baird.         |
| Introvert as long or longer than body. Rows of hooks 12—90.<br>7 coils to gut. West Indies<br>Introvert as long as half-body. Rows of hooks 30. 12—15 coils to gut. Philippines | 20. Nigrescens, Kef.             |
|                                                                                                                                                                                 | 21. Lacteam, Sluit.              |
| Very numerous rows of hooks.<br>Hooks with a curved line on the flattened side dividing the lateral area into two parts                                                         | 22. Duplicigranulatum, Sluit.    |
|                                                                                                                                                                                 | 23. Scolops, Sel. and de Man.    |
| Hooks not bent through a right angle.                                                                                                                                           | 24. Japonicum, Grube.            |
|                                                                                                                                                                                 | 25. Agassizi, Kef.               |
| Few rows of hooks, and hooks without the lateral line on the side                                                                                                               | 26. Granulatum, F. S. Leuck.     |
|                                                                                                                                                                                 | 27. Spengeli, Sluit.             |

## PART IV.

## Geographical Distribution.

The genus *Phymosoma* contains considerably more species than any other genus of the unarmed Gephyreans with the exception of *Phascolosoma*. Including the new species described by Sluiter, and by Selenka in his report upon the Gephyrea collected by the "Challenger," the former genus comprises twenty-seven species, the latter twenty-five. Next to these comes *Aspidosiphon* with seventeen, and *Sipunculus* with sixteen.

Of the twenty-seven species of *Phymosoma* which have been described, seventeen are found in the Malay Archipelago; of these seventeen, thirteen have been found there alone, whilst four have a wider distribution. Three species are found in the West Indies, of which two are found nowhere else; five species in the Red Sea, of which two are peculiar; and four species in the Mauritius, all of which occur elsewhere.

It will thus be seen that the Malay Archipelago is the headquarters of the genus, nearly two thirds of the number of species composing the genus being found there, and nearly one half of the whole number being confined to that region. This is very possibly partly due to the fact that this region of the world is much visited by collectors, and its shore fauna is probably better known than that of any other considerable area within the tropics. On the other hand, the great predominance of the species in these seas is undoubtedly striking.

The following four species have a somewhat remarkable distribution:

(i) *Ph. japonicum*.—This extends along the Japanese coast, and is again met with in the Fiji Islands and off the coast of Australia. It was one of the two species brought home by the "Challenger," and was found by that expedition at Port Jackson. It thus has a considerable north and south distribution. On the other side of the Pacific we find another species,—



(ii) *Ph. Agassizii*, which, while it occurs as far north as the former species, reaches very much farther south. This species stretches from Vancouver's Island down the west coast of America as far as Puntarenas in the Straits of Magellan, and has been found at the intermediate points of San Francisco and Panama. The third species, with a somewhat unusual distribution, is—

(iii) *Ph. Lovénii*, which is found only in the Bergen Fiord. This is still further removed from the equator than the southernmost point reached by *Ph. Agassizii*, but it must be remembered that the Gulf Stream keeps the water on the west coast of Norway comparatively warm. Finally, we find one species,

(iv) *Ph. granulatum*, inhabiting the Mediterranean, and stretching out into the Atlantic as far as the Azores.

If we except the four species whose geographical distribution is described above, the whole genus is confined, with the exception of *Ph. antillarum*, which extends to Puntarenas, between the tropics, or only ventures just beyond them.

The species just mentioned has a somewhat curious distribution; it occurs all round the West Indian Islands, as well as at Surinam and Puerto Cabello; it then crosses over the Isthmus of Panama, and is found along the coast of Chili and Puntarenas. Another West Indian species, *Ph. pectinatum*, is found on the west coast of America, and turns up again at Mauritius, but has not been described from anywhere else. Finally, *Ph. pacificum* has a wide range, stretching from the Red Sea by the Mauritius and India to the Malay Archipelago, and thence to the Philippines and the Fiji Islands; and *Ph. scolops* has a very similar range, occurring in the Red Sea, at Singapore, at the Philippines, and also off the Mozambique coast.

With regard to the bathymetrical distribution of the members of this genus there is little to say; they all live in shallow water, and the greatest depth which I have seen mentioned in connection with them is fifty fathoms.

It is not possible to arrive at any very satisfactory results

from the scanty material at our disposal, with reference to the geographical distribution of this Gephyrean. Nevertheless, so little has been done with regard to the distribution of the lower marine invertebrates, that it seemed to me to be worth while to put together what is known about the occurrence in space of the genus I have been lately working at. The most striking deductions from the facts before us are—(i) the importance of the Malay Archipelago as the headquarters of the genus, but this is possibly more apparent than real; (ii) the restriction, with few exceptions, of the genus to tropical seas; and (iii) their preference for shallow waters. The last two generalisations are obviously connected with the fact that the animals only flourish in comparatively warm water.

In conclusion, attention may be drawn to the association of these animals with coral islands. This may be accidental, and due to conditions of temperature only; but, on the other hand, several species make their homes in tubular holes burrowed out in the soft coral rock.

THE MORPHOLOGICAL LABORATORY;  
CAMBRIDGE, July, 1890.

## DESCRIPTION OF PLATE XI,

Illustrating Mr. Arthur E. Shipley's paper "On a New Species of Phymosoma, with a Synopsis of the Genus, and some Account of its Geographical Distribution."

FIG. 1.—A view of *Phymosoma Weldonii*, enlarged 3 diameters. The introvert with its conical papillæ is slightly protruded.

FIG. 2.—A conical papilla from the introvert, seen from the side and from above.

FIG. 3.—Some of the depressed oblong papillæ from the trunk, enlarged to show the pore and the cuticular plates.

FIG. 4.—A view of the animal cut open just to the right of the middle ventral line. The introvert is retracted. The ventral nerve-cord is seen running up the introvert and back close to the cut edge; the right and left retractor muscles and the two kidneys lie on each side of the coiled intestine. The kidneys are much elongated, and show irregular swellings. The heart with its diverticula is seen in places. The longitudinal muscles of the body-wall are not indicated. Enlarged  $2\frac{1}{2}$  diameters.

FIG. 5.—A diagrammatic view of the head of *Phymosoma Weldonii*. The collar is completely expanded, and surrounds the head. Part of the outer limb of the lophophore involving about ten tentacles has been removed in order to show the pigmented area within the lophophore, and the inner circle of tentacles. The lower lip surrounds the mouth, and at its dorsal end fuses with the end of the lophophore. The dorsal side of the tentacles, which are fully expanded, is pigmented. The lophophore is represented too flat; it should be oblique and overhanging the mouth.

FIG. 6.—A surface view of a piece of the wall of the kidney, showing the glandular areas—crypts—separated from one another by muscle-fibres.

FIG. 7.—An enlarged view of the end of the coiled intestine, with the heart partially dissected out. The spindle muscle running up the axis of the coil is shown near its termination by the anus. The anterior ends of the kidneys are seen right and left.

FIG. 8.—A transverse section through the base of the lophophore, showing the lower lip, the mouth, some isolated tentacles, the fused bases of others, and their blood-vessels and nerves. The fusion of the dorsal ends of the lower lip and of the lophophore, and the distribution of the pigmented and ciliated epithelia are seen.

FIG. 9.—A transverse section through a trunk papilla. This shows some circular muscle-fibres, the ectodermic epithelium passing into the gigantic

excretory cells. Some of the latter contain crystals, others large granules. Only that part of the cuticle which is modified to form the horny plates is shown. Between the plates and round the pore are pigment granules.

FIG. 10.—A transverse section through the skin of the introvert and the ventral nerve-cord. The introvert is retracted so that the outer surface is concave, the inner convex. The section shows the conical papillæ, the thick cuticle with pigment granules, the single layer of ectoderm-cells, the continuous layer of circular and longitudinal muscles, the latter broken only for the insertion of the mesentery supporting the ventral nerve-cord; and the peritoneal epithelium. The nerve-cord shows the dorsal disposition of the nerve-fibres and the ventral ganglion-cells. Some of the secondary nerves are cut as they leave the cord and traverse the mesentery.

FIG. 11.—A transverse section through both the external and internal openings of the nephridium. The structure of the skin is shown, and four of the circular nerves arising from the ventral nerve-cord are seen. The outer wall of the nephridium is fused with the integument, but becomes free posteriorly. The section does not show the whole of either opening, as they do not lie wholly in one plane.

FIG. 12.—A section through the integument.

Mr. Wilson, of the Cambridge Scientific Instrument Company, has drawn Figs. 1 and 5, and Figs. 4 and 7 are drawn from sketches made by Prof. Weldon in Bimini.



## On the British Species of Crisia.

By

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With Plate XII.

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THIS paper will be followed by a further memoir, which will treat of the development of the ovicells and of the embryos in *Crisia*. I have already published a preliminary note<sup>1</sup> on this subject, and I hope to be able to complete the preparation of the more detailed paper without much delay.

It has often been pointed out that the subdivision of the Cyclostomatous Polyzoa into genera and species is attended with peculiar difficulties. The character of the zoœcia remains remarkably constant throughout this group, the systematic study of which is not facilitated by the presence of subsidiary structures, such as the opercula, avicularia, and vibracula, which in the Cheilostomata form so valuable a means of distinguishing the species.

The task of finding satisfactory specific characters within the limits of the genus *Crisia* is not less difficult than in other genera of Cyclostomata, as is seen clearly enough by examining the numerous works which have already been devoted to this genus. Smitt, for instance, in his critical analysis of the Scandinavian forms, has asserted that the delicate *C. geniculata* is connected by a continuous series of intermediate forms with the coarse form which he calls *C. denticulata*, and which he regards as the extreme point which has

<sup>1</sup> 'Proc. Cambridge Philosoph. Soc.,' vol. vii, part 2, 1890, p. 48.

been reached in the evolution of the genus. In his later works he consistently refers to the latter "species" as *Crisia eburnea*, forma *denticulata*, and is of opinion that the "species" recognised by other authors are, for the most part, merely partially fixed points in a continuous series. Most other writers, on the contrary, regard these forms as so many distinct species.

In many of the characters used for distinguishing the several species of *Crisia* from one another—such as the mode of branching, the number of zoëcia in an internode and their individual shape, the position of the ovicells, &c.—each species may vary within wide limits about a certain average. The most satisfactory specific characters appear to me to be furnished by the ovicells; and in this respect I am only confirming the results previously arrived at by Waters<sup>1</sup> for other *Cyclostomata*. Indeed, I believe that in many cases the species cannot be certainly identified unless ovicells are present. Unfortunately, in the great majority of works referring to *Crisia*, the information given with regard to these structures is of the most unsatisfactory character. Many writers, for instance, have mentioned the existence of "pear-shaped" ovicells in certain species; but this character is of generic much more than of specific importance, and the same remark might be made with regard to many of the other characters which have been ascribed to the different species.

The importance of the form of the aperture of the ovicell, as a specific character, has almost entirely escaped the notice of previous writers.<sup>2</sup> Busk<sup>3</sup> has merely stated that the existence of a tubular aperture on the ovicell is a generic character of *Crisia*.

<sup>1</sup> "Ovicells of Cyclostomatous Bryozoa," 'Linn. Soc. Journ. Zool.,' vol. xx, p. 275, and in other places.

<sup>2</sup> Waters has, however, called attention to the importance of this character in several works. See 'Quart. Journ. Geol. Soc.,' vol. xl (Nov., 1884), p. 676.

<sup>3</sup> "Report on the Polyzoa," Second Part, "Challenger" Rep., Zool., vol. xvii, part 50, p. 2.

A comparison of the ovicells (and especially of their apertures) of various forms of *Crisia* has led me to the conclusion that the British fauna includes more species of that genus than are usually recognised. Although the constant occurrence of a particular form of ovicell might possibly be explained by the assumption of a definite correlation between the variations of the zoœcia and of the ovicells (the ovicell being regarded as a modified zoœcium), I do not think that this would give a sufficient explanation of the facts. I find, indeed, that the essential characters of the ovicells are extremely constant, in spite of the occurrence of variations of no inconsiderable magnitude in other parts of the colony.

The following specific diagnoses, which are necessitated by the results which I have arrived at, have been drawn up on the model of those given by Hincks in his well-known 'History of the British Marine Polyzoa.' New lists of synonyms appear to me to be also necessary, in spite of the recent appearance of Miss Jelly's admirable catalogue,<sup>1</sup> to which I must express my great indebtedness. My lists do not profess to be more than a selection of those works in which particular species have been described or figured in sufficient detail to make their identification fairly probable. In many cases I have been obliged to give up the attempt to identify the species to which the description refers.

*C. denticulata*, Lamarek. Plate XII, figs. 1—3.

Zoarium large, erect, of rather straggling habit; the average height of well-grown colonies about one inch; the branches well separated from one another, and with very little tendency to curve inwards. Internodes broad and flattened, but usually with a slight convexity running longitudinally along their anterior face, frequently with a double curve of such a character that if the lower part of the internode is convex towards the right side (e. g.) the upper

<sup>1</sup> E. C. Jelly, 'A Synonymic Catalogue of the Recent Marine Bryozoa,' London, 1889.

part is convex towards the left side; in most cases with an odd number of zoœcia, the dominant number of which appears to be 11. Branches arising fairly high in the internode, usually from the 3rd, 4th, or 5th zoœcium of either side; nearly always given off in perfectly regular alternation on opposite sides of the axis. Each internode with an odd number of zoœcia is normally provided with a single branch, while the even-numbered internodes are, with rare exceptions, branchless. Joints of the zoarium and of the rootlets nearly always jet-black, except in the youngest parts of the colony. Basis rami situated very low down on the zoœcium, and appearing as if wedged in between the zoœcium which bears it and the next zoœcium below it on the same side. Zoœcia entirely adnate with the exception of a short portion, of variable length, which bears the aperture, and which is bent forwards; a pointed projection sometimes occurring at the outer and upper angle of the aperture. Ovicell large, always high in the internode, usually near the end of a branch, and, like the zoœcia, more thickly covered with pores than in the other British species; its aperture inconspicuous, not borne on a prominent tube. Rootlets usually with black joints, which occur at more frequent intervals than in *C. ramosa*. (See also measurements on p. 177.)

*C. luxata*.—

- (1) FLEMING.—‘Hist. of Brit. Animals,’ Edinburgh, 1828, p. 540.
- (2) COUCH.—‘Cornish Fauna,’ part iii, Truro, 1844, p. 99, pl. xviii, fig. 3.

*C. denticulata*.—

- (3) H. MILNE-EDWARDS.—“Mém. sur les Crisies,” ‘Ann. Sci. Nat.,’ 2<sup>e</sup> sér., ‘Zool.,’ tome ix, 1838, pl. vii, fig. 1.
- (4) JOHNSTON.—‘Brit. Zoophytes,’ 2nd ed., London, 1847, p. 284, pl. 1, figs. 5, 6.
- (5) CARUS.—‘Prodromus Faunæ Mediterraneæ,’ vol. ii, Stuttgart, 1889, p. 39.

*C. denticulata* (pars).—

- (6) BUSK.—‘Cat. Marine Polyzoa Brit. Museum,’ part 3, 1875, pl. iv, figs. 1—4.
- (7) HINCKS.—‘Hist. Brit. Marine Polyzoa,’ London, 1880, p. 422, pl. lvi, figs. 7, 7a.



*C. eburnea*, Linn. Plate XII, fig. 6.

Zoarium forming dense tufts, usually attached by a single stem, the base of which does not, in most cases, develop many rootlets; the average height of well-grown colonies from  $\frac{1}{2}$  to  $\frac{3}{4}$  inch; the branches characteristically curved inwards. Internodes usually short, somewhat flattened; in most cases with an odd number of zoëcia, the dominant numbers being 5 and 7. Branches generally arising from the lowest zoëcium in an internode, sometimes higher up; one branch is normally developed from each odd-numbered internode, even-numbered internodes being ordinarily branchless. On the main stem or the principal branches, the branches come off in regular alternation on opposite sides: nearer the growing-points, they are arranged in compound helicoid cymes, of the formula<sup>1</sup>—

$$\begin{array}{l} (n + r_1) + \\ \quad | \\ = (n + r_1) + \\ \quad \quad | \\ \quad \quad = (n + r_1) + \\ \quad \quad \quad \&c. \end{array}$$

Joints yellow, or colourless near the growing-points, sometimes becoming dark brown in the older parts of the colony. Basis rami short, not wedged in between two zoëcia. Zoëcia almost entirely adnate, the upper portion, which bears the aperture, free, bent forwards nearly at right angles to the lower part; frequently a conspicuous pointed process on the outer side of the aperture. Ovicell large, curved inwards, usually replacing the second, or, less often, the third zoëcium of an internode; its aperture conspicuous, elongated from side to side, borne on a very distinct tube, which is wider at its base than at its summit. Rootlets usually developed in very small numbers. (See also measurements on p. 177.)

*C. eburnea*.—

(4) JOHNSTON.—P. 283, pl. 1, figs. 3, 4.

(5) CARUS.—P. 38.

<sup>1</sup> This method of representing the branching is explained on p. 146. In the above formula  $n$  would usually be 5, less often 7 or higher numbers.

- (8) SMITT.—“Om Hafs-Bryozoernas utveckling och fettkroppar,”  
‘Öfvers. af K. Vet.-Akad. Förhandl.,’ 1865, No. 1, p. 9, pl. i,  
figs. 15—18.

*C. eburnea* (pars).—

- (9) SMITT.—“Krit. förteckn. öfver Skandinaviens Hafs-Bryozoeer,” I,  
‘Öfvers. af K. Vet.-Akad. Förhandl.,’ 1865, No. 2, pl. xvi, figs.  
10, 11, 13—19.  
(6) BUSK.—Pl. ii, figs. 1, 2 ; pl. v, figs. 1, 2.  
(7) HINCKS.—P. 420, fig. 21 (p. 416).

*C. eburnea*, forma *eburnea*.—

- (10) SMITT.—“Bryozoa marina in regionibus arcticis,” ‘Öfvers. af K.  
Vet.-Akad. Förhandl.,’ 1867, No. 6, pp. 444, 461.  
(11) SMITT.—“Recensio Syst. Bryozoorum Novaja Semlja,” *ibid.*, 1878,  
No. 3, p. 12.  
(12) SMITT.—“Recensio Bry. e mari arctico,” *ibid.*, 1878, No. 7, p. 23.  
(13) FREESE.—“Beschr. Ostsee Bryozoen,” ‘Arch. f. Naturg.,’ 54,  
Jahrg., Bd. i, 1888, p. 31, pl. ii, fig. 18.

*C. aculeata*, Hassall. Pl. XII, fig. 4.

Zoarium of very delicate habit, resembling that of the next species, from which it may be distinguished by its much slenderer appearance; the average height of well-grown colonies from  $\frac{1}{2}$  to  $\frac{3}{4}$  inch, the branches with very little tendency to curve inwards. Internodes usually short, often consisting of five or seven zoëcia; but much longer internodes, with more numerous zoëcia, may occur, especially at the ends of the branches. Branches usually arising from the 1st or 2nd zoëcium of either side of an internode, but sometimes (especially in the case of internodes near the ends of the branches) higher up: an internode (especially a peripheral one) may bear two or more branches. In nearly all colonies, in addition to the ordinary branches, some of the internodes bear long, jointed spines, which are curved inwards over the anterior side of the branch; these spines are most often developed from the lower zoëcia of an internode, or at the apices of the terminal internodes. Joints yellow, or colourless near the growing-points. Basis rami usually short, not wedged in between two zoëcia. Zoëcia with a conspicuous, free, tubular portion, bearing the aperture; this portion is curved forwards,

but not as in the last species; it is usually lost in the zoœcia of the lower parts of the colony. Aperture circular, with no projection on its outer side. Ovicell small, fairly high in the internode, prominent near its upper end, and falling away very suddenly to the aperture, which lies on the surface of the zoœcium next above the ovicell on the same side of the internode; this zoœcium curves round the back of the ovicell, and always acquires a characteristic relation to the aperture of the latter; this aperture is inconspicuous, and is never borne on a distinct tube. Rootlets resembling those of *C. ramosa*. (See also measurements on p. 177.)

*C. aculeata*.—

(14) HASSALL.—“Cat. of Irish Zoophytes,” ‘Ann. and Mag. Nat. Hist.,’ vol. vi, 1841, p. 170, pl. vii, figs. 3, 4.

(15) Supp. to ‘Cat.,’ *ibid.*, vol. vii, 1841, p. 366.

(4) JOHNSTON.—P. 285.

(16) SMITT.—“Bidr. till känn. om Hafs-Bryozoernas utveckling,” ‘Upsala Univ. Årsskrift,’ 1863, p. 3.

Smitt agrees with van Beneden (20) in stating that the ovicells are completely closed.

(17) JOLIET.—“Cont. à l’hist. Bryozoaires, Cotes de France,” ‘Arch. Zool. Exp. et Gén.,’ vol. vi, 1877, p. 286.

*C. eburnea*, var. *aculeata*.—

(6) BUSK.—P. 4.

(7) HINCKS.—P. 421, pl. lvi, figs. 5, 6.

(18) JULLIEN.—“Liste des Bry. rec. à Étretat,” ‘Bull. Soc. Zool. France,’ t. vi, 1881, p. 14.

(19) VINE.—“Rep. on Recent Marine Polyzoa,” ‘Brit. Association Report,’ Aberdeen Meeting, 1885, p. 588.

*C. eburnea*.—

(3) MILNE-EDWARDS.—Pl. vi, fig. 2.

? (20) P. J. VAN BENEDEN.—“Rech. sur l’Anat. . . . Bryozoaires . . . Ostende,” ‘Nouv. Mém. de l’Acad. de Bruxelles,’ t. xviii, 1845, pl. iii, figs. 12—16.

Van Beneden states that the ovicells are closed on all sides; and this statement is more likely to have been made of *C. aculeata* (in which the aperture of the ovicell is very inconspicuous) than of *C. eburnea*. The specimens figured are by no means unlike *C. aculeata*, but they have no spines.

? *C. eburnea* (pars).—

(9) SMITT.—Pl. xvi, figs. 12*a*, 12*b*.

On p. 135 of Smitt's paper it is explained that these figures represent young ovicells (of *C. eburnea*), without tubular apertures. It may, however, be remarked that an ovicell, with the contents shown in fig. 12*b*, would probably have had a well-developed tubular aperture if it had really belonged to *C. eburnea*; and, further, that there is evidence (see above, No. 16) that Smitt has worked at the ovicells of *C. aculeata*. Ibid., table of formulæ, Nos. 2, 3 (see explanation of the formulæ), and probably some of the later formulæ.

? *C. denticulata* (pars).—

(6) BUSK.—Pl. iii, figs. 1—6.

Notice the spine in fig. 6. In the other figures, the character of the branching and of the basal internodes, and the small number of the pores appear to me to prove that this plate does not refer to *C. denticulata*, and that it probably refers to *C. aculeata*.

### *C. ramosa*, n. sp. Pl. XII, figs. 10, 11.

Zoarium erect, often of rather straggling habit; the average height of well-grown colonies about  $\frac{3}{4}$  inch; the branches (in well-grown specimens) arranged in fan-shaped systems, owing to the large number of branches given off by the terminal internodes, and with little or no tendency to curve inwards. Internodes often much flattened, of very variable length; often very long, and consisting of numerous zoœcia; in this case often with a well-marked double curve, as in *C. denticulata* (and, to a less extent, in other species). Branches developed in greater numbers than in any of the other British species; even in the lower parts of the colony the internodes commonly bear two branches, while the terminal internodes, and especially those which bear ovicells, may give rise to as many as four or five branches, which do not necessarily come off alternately on opposite sides of the stem. The lowest branch of an internode very commonly comes off from the second zoœcium of one side; if the lowest branch arises from the first zoœcium of the internode, the next branch is usually given off by the third zoœcium of the opposite side. Joints yellow, or colourless near the growing-points, never black. Basis rami long, usually reaching the aperture of the



zoëcium next below it on the same side, unless it is borne by the lowest zoëcium of an internode. Zoëcia usually with a long, free, tubular portion bearing the aperture; this portion is distinctly curved forwards, but is usually lost in the older parts of the colony; in other cases this tubular portion is not developed to more than a very slight extent. Aperture circular, without any pointed projection on its outer side. Ovicell very large, and more regularly pear-shaped than in any of the other species; usually a little higher in the internode than in *C. aculeata*, but in some cases it may occupy as low a position as that of the fourth member of the internode; it is perhaps most commonly in the position of the 6th—8th member; its aperture circular, borne on a long and very conspicuous funnel-shaped tube, which is considerably wider at its summit than at its base. Rootlets often developed in considerable numbers, sometimes attaining a great length (nearly an inch), and composed, for the most part, of long segments, separated by yellow or colourless joints. (See also measurements on p. 177.)

(The following list includes references to several forms of *Crisia* which, as explained below, I do not believe to be identical with *C. ramosa*.)

? *C. cribraria*.—

- (21) STIMPSON.—“Synopsis of the Marine Invertebrata of Grand Manan [Bay of Fundy],” ‘Smithsonian Conts. to Knowledge,’ vol. vi, 1854.

This species may be identical with *C. ramosa*, in which case my own specific name will have to be given up. The zoëcia are described as being “so crowded as to form often two or three longitudinal rows, in which they are usually opposite” (p. 18). I do not see how such a statement could be made of *C. ramosa*. The figures given (pl. i, figs. 8*a*—*c*), although not unlike that species, are not drawn with sufficient care to enable a satisfactory conclusion to be arrived at.

? *C. arctica*.—

- (22) M. SARS.—“Geol. og Zool. Jagtt. anst. p. en Reise Trondhjems Stift.,” Christiania, 1863.

The zoarium of this form is said to reach the height of 30 mm.; the branches and the zoëcia are straight, or nearly straight; the internode possesses, on each side, two to three, often eight to twelve, rarely twenty to twenty-one zoëcia. The species is said to resemble *C.*

denticulata and *C. cribraria*. It differs, according to Sars' description, from my own specimens in the following respects.

The zoecia are fused with one another along their whole length, so that the upper part, with the aperture, is not free. The outer and upper angle of the young zoecia may bear a small knob (never observed in *C. ramosa*). The joints are usually uncoloured, but sometimes brown-grey in the older branches (usually yellow in *C. ramosa*). The ovicells are always in the axils of the branches, and they are not described as having an aperture (which can hardly be overlooked in *C. ramosa*).

On the whole, Sars' description suggests a form like *C. denticulata* or *C. elongata*, M.-Edw. It is, perhaps, the form figured by Smitt (9) in pl. xvi, fig. 20. The "basis rami" in this figure is unlike anything I have ever seen in *C. denticulata*, although resembling that of *C. ramosa*.

*C. eburnea* (pars).—

(2) COUCH.—P. 99.

Some of the larger specimens mentioned by Couch probably belonged to this species: the ovicells are "somewhat urn-shaped with narrow tubular necks, which are not placed in the centre." This description probably refers to *C. ramosa*, although the "young specimens" in which the branches "all arch inwards" doubtless belonged to *C. eburnea*. The magnified figure (pl. xviii, fig. 2), which is not good, may be identified as *C. aculeata* by the presence of a spine; and the figure next to it (natural size) is probably either that species or *C. ramosa*.

? *C. eburnea* (pars).—

(9) SMITT.—Pl. xvi, fig. 9, and p. 135 (fig. 6). (These figs. may refer to *C. aculeata*.)

? *C. eburnea*, var.—

(6) BUSK.—Pl. v, figs. 5—10.

? *C. denticulata* (pars).—

(6) BUSK.—Pl. ii, figs. 3, 4.

(9) SMITT.—'Table of Formulæ,' Nos. 14—17, and probably some of the earlier numbers (e.g. 12 and 13), which are said to belong either to *C. eburnea* or to be transitional from this form to *C. denticulata*. It is hardly possible that a form with so many branches arising from the same internode as in No. 17, for instance, was really *C. denticulata*.

(23) SMITT.—"Floridan Bryozoa," Part 1, 'Kongl. Svenska Vet.-Akad. Handl.,' B. x, No. 11, 1872, pl. i, figs. 1—5.

I do not feel certain that the form described in (23) is really identical with *C. ramosa*, although it can hardly be regarded as *C.*

denticulata. The form of the zoëcia is very similar to that found in *C. ramosa*; but, on the contrary, the ovicells do not agree with those of the latter. If the left side of fig. 5 represents a young ovicell (probably somewhat broken), the ovicells are even less like those of *C. ramosa* in their early than in their fully developed condition. Is this form possibly identical with the one described by Stimpson (21) under the name of *C. cribraria*?

(7) HINCKS.—P. 423.

The statement that the ovicells of *C. denticulata* have "a tubular orifice at the top" was possibly made after an examination of *C. ramosa*; especially as, on the same page of Hincks's work, occurs *C. denticulata*, var. *α* (to which pl. lvi, fig. 9, presumably belongs); and there can be little doubt that this is really *C. ramosa*.

*C. denticulata*, var. *tenuis*.—

(24) VIGELIUS.—"Cat. of the Polyzoa ... Willem Barents," 'Nied. Arch. f. Zool. Supplementb.,' i, 1881-2.

This form is said to correspond closely with Hincks's unnamed variety just referred to. It is, however, impossible to accept *tenuis* as a specific name, since the name *C. tenuis* had been applied by MacGillivray to an Australian species before the appearance of the paper by Vigelius (see F. McCoy, "Prodromus of the Zool. of Victoria," 'Decade' iv, pl. xxxix, Melbourne, 1879.

? *C. fistulosa*.—

(6) BUSK (non Heller).—P. 5, pl. vi A, figs. 1, 2.

Even if this form is identical with the species under consideration it is better to drop Busk's name, since the specific name *fistulosa* was originally applied by Heller to a form which is clearly not the one described by Busk (see Waters, No. 25).

Through the kindness of Mr. R. Kirkpatrick I have been enabled to refer, at the British Museum, to a specimen of the form described by Busk; and I have also to thank Mr. Kirkpatrick for having subsequently given me further information on the same subject. The specimen in question is labelled "*C. fistulosa*, Hell., locality unknown. Lesina?" I am informed by Mr. Kirkpatrick that the label is in Mr. Busk's handwriting, with the possible exception of the last word; and that the specimen is probably really from the Mediterranean.

The specimen in the British Museum is even more like my own species than is obvious from Busk's description, which, in Mr. Kirkpatrick's opinion, was probably taken from that specimen. As many as five branches may come off from the same internode, and some of them higher than the sixth zoëcium, which, according to Busk, is their upper limit. The ovicells, of which only two could be satisfactorily examined, agree fairly well with those of the Plymouth form. Their

diameter is about .45 mm. Mr. Kirkpatrick further informs me that the distance from aperture to aperture is .4 mm., and that the total length of the zoëcium is about .7 mm. Although these numbers are distinctly smaller than the average measurements of corresponding structures in *C. ramosa*, I am inclined to believe that my own specimens belong to the same species as the one in the British Museum.

Waters (25), in 'Ann. and Mag. Nat. Hist.,' 5 ser., vol. iii, 1879, p. 269, pl. xxiii, fig. 4 ("Bryozoa of the Bay of Naples"), identifies *C. fistulosa*, Busk, with what he calls *C. elongata*, var. *angustata*. I cannot, however, believe that *C. ramosa* is identical with the form described by Waters. Although the number of zoëcia in the internode in *C. ramosa* may be large, this species could hardly be characterised as having fourteen to twenty-six zoëcia in the internode; nor does the description, "branches arising usually from the fifth to eighth zoëcium of a branch, and at about the same distance a fresh branch grows on the other side," correspond with the branching of *C. ramosa*. As Mr. Kirkpatrick has pointed out to me, Waters' statement that the zoëcia are .04 mm. apart was no doubt due to an oversight.

For further remarks on *C. fistulosa*, Busk, see Vine (19), p. 589.

The characters of the ovicell are so constant in my specimens that, taken in conjunction with other facts, I cannot resist the conclusion that this form deserves recognition as a species. Although it is obviously alluded to in some of the works just quoted, I cannot identify it with certainty with any form which has hitherto received a specific name; and I therefore suggest for it the name *C. ramosa*, in allusion to the large number of the branches given off by a single internode.

*C. ramosa* has been found in large numbers at Plymouth, where it is certainly the commonest of all the forms of *Crisia*.

While the identification of fully developed colonies of *Crisia*—in those cases at least where ovicells are present—cannot often be a matter of doubt, it may be extremely difficult to identify the species to which a small fragment of a colony or a young zoarium belongs. The greatest difficulty is found, in these cases, in distinguishing *C. eburnea* from *C. aculeata*, or the latter species from *C. ramosa*. The characters of the several species can be best brought out by a careful comparison, under a series of distinct heads, of their more obvious external features.



**Habit of Zoarium at Different Seasons ; Regeneration.**

A very slight acquaintance with the British forms of *Crisia* enables one to distinguish at a glance, in most cases, the species to which a given specimen belongs. *C. denticulata* is characterised by the coarseness of its general habit ; by the regular dichotomous appearance of the branching, as seen by the naked eye ; and by the fact that the branches diverge from one another to such an extent that they are separated from one another by considerable interspaces at their ends. In *C. eburnea* the branches are inflected towards the axis of the colony, and are so closely massed together that it is impossible to study the exact character of the branching without first disentangling the branches. On flattening the specimen out on a slide the cymose character of the branching is at once apparent. *C. aculeata* possesses a characteristic delicacy of habit ("of a slenderer habit than *C. eburnea*, which the species closely resembles"<sup>1</sup>) ; and it may be compared, in external form, to a *C. eburnea* which has become of much laxer and slenderer habit than usual, and in which comparatively few branches have been developed. The branches are much straighter than in *C. eburnea*. *C. ramosa* is extremely similar, in general appearance, to *C. aculeata*, but is of distinctly coarser habit ; the branches are very straight, and the number of the branches to which the internodes near the growing-points give rise results, in actively growing colonies, in the formation of fan-like systems of branches. The long tubular apertures of the zoæcia (if developed) give a characteristic appearance to the species, which cannot, however, in all cases be distinguished by the naked eye from *C. aculeata*.

The above remarks apply especially to colonies in their fully developed condition ; but the appearance of any species depends largely on the time of year at which it was found. Many of the specimens of *C. eburnea* found in the early spring are provided with numerous ovicells, the ultimate fate of which seems to have hitherto attracted no attention, although

<sup>1</sup> Johnston, G., 'A Hist. of the British Zoophytes,' ed. 2, p. 286.

there can be no doubt that these structures disappear after the end of the breeding season. I have looked in vain for any signs of the absorption of the ovicells in *Crisia*; and the following facts probably imply that they are simply thrown off from the colony after the liberation of the embryos which have been produced in them.

The typical spring form of *C. eburnea* possesses a conspicuous main stem, which forms an obvious central axis, from which the rest of the colony comes off as a series of branches, developed in regular alternation on opposite sides, and decreasing in size fairly regularly from the base to the summit of the colony. The main stem consists of perhaps eleven or twelve internodes, each of which normally gives rise to a branch; and the branches themselves are usually provided with a profusion of ovicells, many of which are still in process of development, and most of which are near the ends of the branches.

In a colony of the same species found in May most of the ovicells were at some distance from the ends of the branches, owing to the development of several (7—8) zoœcia above the ovicells; and the branches which bore ovicells had, in most cases, completely finished their growth: very few ovicells, and these of a weakly appearance, were being developed. Most of the branches ended in slender internodes, in which growth was no longer taking place, as was shown by the fact that no growing-points were left. The exhaustion of the colony was further shown by the fact that some of the terminal internodes consisted of no more than two or three zoœcia, with no growing-points.

In the summer (August) large and highly branched colonies with active growing-points are found, but they are normally without any trace of ovicells. In many of these cases it may be noticed that the main stem has been broken, and is merely represented by its basal portion. The rest of the colony will, in this case, probably consist of a small number of large branches given off from the remains of the stem or of its lateral branches, and in many cases the sharp contrast between

the clean white appearance of these highly branched parts of the colony and the dirty-brown appearance of the stump of the main stem, covered as it is by foreign growths of various kinds, will give rise to the suspicion that the former have been developed at a later period than the latter, and that the latter are the remains of colonies which developed ovicells at an earlier period of the year.

Smitt<sup>1</sup> has called attention to the fact that the free tubular portion of the zoëcium of *C. geniculata* is sometimes relatively transparent, and that it is separated by a sharp line from the basal, more highly calcified part, and he suggests that this transparent portion has in these cases been regenerated. He further points out<sup>2</sup> that in *Aetea argillacea* (= *Aetea truncata*, forma *abyssicola elongata*<sup>3</sup>) this process of regeneration seems to be periodic, since a zoëcium consisting of portions of three different ages was in one case observed by him; and that in *Farrella fusca* (= *Vesicularia fusca*<sup>4</sup>) the zoëcium may attain twice its normal length by the occurrence of this regenerative process.<sup>5</sup> Milne-Edwards<sup>6</sup> had previously pointed out that the zoëcia were able to form rootlets at an advanced period of their existence.

There can be little doubt that Smitt's suggestion is a correct one. In *C. eburnea* the older parts of the colony are frequently covered with an encrusting red seaweed, the presence of which has no doubt been responsible for the "rose-red" colour which has been mentioned by Johnston<sup>7</sup> and others as a feature which sometimes characterises the species. In certain specimens found in April the basal parts of the colonies were completely covered by this encrusting growth, while in various

<sup>1</sup> 'Öfvers. af K. Vet.-Akad. Förhandl.,' 1865, No. 2, p. 128.

<sup>2</sup> "Om Hafs-Bryozoernas utveckling och fettkroppar," 'Öfvers.,' &c., 1865, No. 1, pp. 29, 30.

<sup>3</sup> 'Öfvers.,' &c., 1867, No. 5, p. 280.

<sup>4</sup> 'Öfvers.,' &c., 1866, pp. 502, 505.

<sup>5</sup> *Ibid.*, pl. xiii, fig. 39, and explanation of figure.

<sup>6</sup> 'Ann. Sci. Nat.,' 2<sup>e</sup> sér., "Zool.," tom. ix, 1838, p. 196.

<sup>7</sup> 'British Zoophytes,' ed. 2, p. 284.

parts perfectly white growing points or new apertures were making their appearance. Similar phenomena of regeneration have been repeatedly observed in all the species which I have examined. Thus the first glance at an ordinary colony of *C. ramosa* will suffice to show that the tubular ends, so characteristic of the young zoëcia, are absent in the lower parts of the colony, where they have been either broken off or absorbed. The zoëcia which are in this condition are closed by an obliquely placed diaphragm, as described in *Crisia* and other *Cyclostomata* by Waters,<sup>1</sup> Pergens,<sup>2</sup> and others. On staining a specimen of *C. ramosa* without decalcification, it is at once obvious that these diaphragms are used for the closure of zoëcia which contain brown bodies but no functional polypides. They are placed at the point where the zoëcium normally becomes free from the internode, and the free portion becomes gradually broken away or absorbed down to the point where the diaphragm is situated. In the younger parts of the colony, where the zoëcia possess free tubular ends, no diaphragms are present, and functional polypides or obvious buds, together with the brown bodies formed by the death of the last polypides, are found in nearly all the zoëcia.

The individual life of the zoëcium has not, however, necessarily come to an end with the formation of one of these diaphragms, as may be easily proved by the examination of suitable spring colonies which have been stained with borax carmine without decalcification. Whilst zoëcia in which no regeneration is taking place are closed by a diaphragm and appear perfectly unstained, the red colour of the regenerating parts is obvious at the first glance. The first indication of the renewed activity of a zoëcium is given by the fact that some of the cells below the diaphragm have acquired the power of

<sup>1</sup> A. W. Waters, "Closure of the Cyclostomatous Bryozoa," 'Linn. Soc. Journ. Zool.,' vol. xvii, 1884, p. 400; "Fossil Cyclostomatous Bryozoa from Australia," 'Quart. Journ. Geol. Soc.,' vol. xl, 1884, p. 675.

<sup>2</sup> Ed. Pergens, "Revision des Bryozoaires du Crétacé figurés par d'Orbigny," 1<sup>re</sup> partie, "Cyclostomata," 'Bull. de la Soc. Belge de Géol.,' &c., tome iii, 1889, p. 317.



taking up colouring matters; slightly later a young polypide bud is seen below the diaphragm, which is then absorbed, the zoecium growing out (in *C. ramosa*) into a long tubular portion, at the end of which is the aperture.<sup>1</sup> In *C. ramosa* the free portions of regenerated zoecia are sometimes considerably longer than the normal length of the tubular portion. In one case the regenerated portion, which was completely free from the branch, was .69 mm. long.

It is well known that new stems are given off from various parts of the rootlets.<sup>2</sup> These rootlets are usually developed from the backs or sides of the zoecia, especially of those near the base of the colony. But in cases where regeneration is actively taking place the tip of a branch may grow out into a rootlet, or a rootlet may take the place formerly occupied by a zoecium, usually one of the terminal zoecia of an internode in this case.<sup>3</sup> The rootlet thus formed may grow for a considerable distance, and finally produce a new stem as a lateral branch; or the new stem may be the actual prolongation of the rootlet, which, after a longer or shorter course, assumes the characters of a stem. In other cases a new growing-point is formed from an old joint at the point where a lateral branch or an axial internode has previously been lost; or it may be formed from the apex of an internode in which the fracture has taken place across the middle of the internode, instead of at an axial joint. The result of this is that it is very common to observe an old brown stem from which start new internodes (lateral or axial), which are shown, by reason of the perfectly white appearance of their ectocyst, to have been formed at a much later period than the brown part of the stem. In one or two cases a growing-point had started from the proximal side of a broken joint, and had then given rise to a stem which grew in a direction directly opposite to that of the internode from which it was developed. These cases are somewhat analogous to the

<sup>1</sup> This is the process which was observed by Smitt in *C. geniculata*.

<sup>2</sup> Cf. Smitt, "Krit. Fört.," i, 'Öfvers.,' &c., 1865, p. 122.

<sup>3</sup> These statements refer, for the most part, to *C. eburnea* and to *C. ramosa*.

one described by Smitt,<sup>1</sup> in which a "basis rami" had given rise to a normal branch, and also to a growing-point directed straight downwards from its base, which was formed by the proximal end of the "basis rami," from which it was separated by a joint.

Although regenerated lateral branches may start from the old lateral joints, it is not uncommon to find that they are given off from near the end of the old internode, instead of in their normal position lower down; this is due to the fact that the aperture of an old zoëcium has become a growing-point.

In colonies in which the process of regeneration is commencing, it is frequently noticed that the young growing-points are appreciably smaller than the normal ones. These small growing-points naturally give rise to slender zoëcia and branches, which, however, as they grow longer, acquire fresh strength, and soon regain their normal diameter. The regenerated parts of a colony are, consequently, often joined to the older parts by slender bases, in which, moreover, the basal internodes may consist of an unusually small number of zoëcia. In both these respects they resemble colonies which are developed directly from the larva, or from a growing-point which starts from the rootlet of an old colony.

It is important to notice that, so far as my observations go, the regenerated parts of a colony always retain the same specific characters as the older parts. I have looked in vain for any indications which might have been given by regenerating colonies that the forms of *Crisia* described above as distinct species might be merely different phases of the same species.

The general life-cycle of *C. eburnea* may probably be summarised as follows:—The breeding season is at its height in April and May; and at about this period it is not difficult to find young individuals which consist of a single zoëcium attached by a disc-like base, and which have resulted from the metamorphosis of a free larva; small colonies are soon formed by these primary zoëcia. At first, rootlets may be altogether absent, and in many colonies they are developed very sparingly;

<sup>1</sup> Loc. cit., p. 125.

but when formed, some of them give rise to fresh stems, which are the starting-points of new colonies. Or, again, some of the specimens found in the summer have resulted from colonies which developed ovicells in the earlier part of the year, and which, after losing these structures, again burst out into renewed growth; in some cases leaving a single ovicell on the colony as some indication of their past history. In the spring, ovicells, when present at all, are found in large numbers, and those well-developed colonies which do not possess them at this period are probably in most cases of the male sex. Thus, in order to find spermatozoa in April, it was generally quite sufficient to select any colony in which there were no ovicells, while spermatozoa were not discovered in any of the cases in which ovicells were present.

In the early spring the discoloured stumps of colonies which grew during the preceding year are found; from various parts of these, new growing-points are developed, and give rise to the colonies found at a slightly later period. The production of the enormous number of embryos then developed seems to exhaust the energy of the colony, whose growth practically ceases for a time, many of the branches being thrown off. After a period of rest, growth recommences with great vigour, and by the middle of the summer large and highly branched colonies are again found, although now, as a rule, with no ovicells.

#### Number of Zoëcia in the Internode, Mode of Branching, &c.

Most of the previous accounts of *Crisia* merely mention the limits between which the number of zoëcia in the internode may vary in the several species. Thus Hincks<sup>1</sup> says of *C. eburnea*, "3—9 cells in an internode;" while Johnston<sup>2</sup> is a little more explicit, stating that "there are from two to five, sometimes seven, and very rarely even nine, cells in each internodal space" in the same species. It appears to me that it is quite impossible to define accurately the several species

<sup>1</sup> 'British Marine Polyzoa,' p. 421.

<sup>2</sup> 'British Zoophytes,' ed. 2, p. 284.





—representing the axis of a branch given off by the main stem of a colony, together with all the ramifications of two of its secondary branches.

It will be noticed that nearly every internode develops a single branch, and that the branches come off in regular alternation on opposite sides of consecutive internodes of every axis. Although the number of zoëcia in the internode is very variable, eleven may be regarded as the number most characteristic of the species.

The above formula further shows that every branch-bearing internode whose development is complete possesses an odd number of zoëcia, while in completely developed internodes which bear no branch the number is even. Although this rule is not quite absolute, it is difficult to find any exception to the striking rule that a branchless internode has an even number of zoëcia; or, conversely, that an internode with an even number (whether this number is large or small) of zoëcia bears no branch. It may be pointed out that even if the branches have been broken off, their previous presence can be ascertained by the existence of the basal articulation from which they formerly sprang.

It may further be noted that a lateral branch is, with very rare exceptions, produced on the side of the basal zoëcium of the internode (Pl. XII, fig. 2).

The regular alternation of the zoëcia of the axis of any branch is not disturbed by the development of an axial joint; and the last zoëcium of the internode below the joint nearly always projects beyond the penultimate zoëcium (which belongs to the other side) in the form of a free tube (fig. 2). Since the branch-bearing internode has an odd number of zoëcia, and since the branch is developed on the side of the basal zoëcium, it follows that the last zoëcium, which is produced into a free tube, will also be on the same side as the branch. A moment's consideration will show that the basal zoëcium, the branch, and the terminal ("produced") zoëcium, in any internode, will normally be on the opposite side to that on which these structures are situated, both in

the internode below it and in the internode above it on the stem.

But if an even-numbered internode is developed (fig. 2), its last zoëcium will of course be on the same side of the stem as the last zoëcium of the preceding internode; and consequently the basal zoëcium and the branch of the internode above it will be on the same side as its own basal zoëcium, and on the opposite side to the branch next below it; or, illustrating this by a formula, we shall, as a rule, find cases like  $(13 + r_5) + (8) + (7 + r_2)$ , as shown in fig. 2.

Thus, stating the same fact in another way, an even-numbered and branchless internode may be intercalated in the stem without disturbing the alternate origin of the branches on opposite sides. The same is true of those cases where two even-numbered internodes occur consecutively on the same axis.

The more closely one investigates unusual methods of branching in this species, the more obvious does it become that the growth of the colony is regulated by some well-defined law, which finds one of its expressions in the preceding rule.

Thus it will be seen, by reference to figs. 2, 4, 6, and 11, that the basal zoëcium of a lateral branch is on the abaxial side of the latter in all the four species referred to; and further, that the branch given off by the basal internode of an axis is also on the abaxial side. This is obvious enough for *C. denticulata*, from the formula on p. 146, where it will be noticed that, in the one case in which the basal internode has an even number of zoëcia, the second internode develops the first branch, and that that branch (and of course the basal zoëcium) is on the abaxial side.

On two occasions abnormal branching of the type  $(9 + r_3) + (13) + (11 + r_3)$  was noticed. Here an odd-numbered branchless internode occurs; but such cases seem to be rare. Since the number of zoëcia in the branchless internode is odd, it follows that the basal zoëcium, and consequently the branch, of the third internode will be on the same side as in the first internode.



internodes have an odd number of zoëcia, and in which, further, the branch and the basal zoëcium are, in each internode, on the same side. The third internode which is completely figured has 10 zoëcia, and possesses no branch. It must, however, be pointed out that in his fig. 1 *a* (*C. denticulata*, under slight magnification) Milne-Edwards represents most of the internodes as having an even number of zoëcia; but it may probably be assumed that in this figure, which gives an excellent representation of the general appearance of the species, sufficient attention has not been paid to the details of the arrangement of the zoëcia. Again, Busk<sup>1</sup> figures, in the same species, two complete internodes, one of which has thirteen zoëcia and a branch, and the next has twelve zoëcia and no branch.

The relations above described are perhaps capable of being, to some extent, explained in the following manner. In the species of *Crisia* which I have examined, and, I have very little doubt, throughout the genus, the base of an internode, whether axial or lateral, is simply the basal part of the lowest zoëcium of that internode, that part having been separated by the development of the joint from its upper or distal part. This will be intelligible on referring to Pl. XII, fig. 1, representing an axial internode in which only two zoëcia are completely separated from the growing-point. The lowest zoëcium of the internode is seen to be divided into two parts by the horny joint; and the lower of these two parts forms the articulation to which the younger internode is attached. In examining the formation of the joint (whether axial or lateral) in stained specimens it is at once obvious that the alimentary canal of the youngest zoëcium of the internode at first extends, through the tubular joint, into this lower portion; confirming the statement made above with regard to the morphology of the base of the internode.

It is thus clear that the occurrence of an axial joint in no ways disturbs the alternation of the zoëcia (see any of the figures). The last zoëcium of the older internode would

<sup>1</sup> 'Cat. of Mar. Pol. in Brit. Museum,' Part III, "Cyclostomata," pl. iv, fig. 2.



overlap, and be fused with the next zoëcium higher up on the same side if it were not for the development of the joint ; which is, however, formed in such a position across a zoëcium as to leave the preceding zoëcium in the characteristic “ produced ” condition which has already been described. In the ordinary type of branching, where successive internodes produce branches in regular alternation on opposite sides, the number of zoëcia must be odd if the branch is to be produced on the side of the basal zoëcium in each internode. The formation of a new axial internode practically amounts to the transverse division of a zoëcium, while the formation of a branch may be expressed as due to the longitudinal division of a zoëcium (at the growing-point). Suppose that the right side of an axis bears a branch (as in the lowest internode shown in fig. 2). The tendency of the growing-point to produce new branches alternately on opposite sides would normally result in the production of a branch from the left side of the next youngest internode ; but if a lateral branch has not been produced by the time that a new axial joint is to be formed, that axial joint would be, as a matter of fact, normally developed from a zoëcium of the left side, as at the base of the third internode in fig. 2 ; and this implies the existence of an even number of zoëcia in the second internode. The production of an even-numbered internode may thus be regarded as due to the alternate predominance of the two sides of the growing-point. The development of a lateral branch on the right side (e.g.) has apparently the effect of leaving the left side of the growing-point with an excess of vigour ; so that when a new internode is formed—whether by the transverse division of a zoëcium to form an axial joint, or by its longitudinal division to form a lateral branch—it is the left side (in this particular case) of the growing-point by which this division is effected. Division in the transverse direction results in the formation of an even-numbered internode, while the production of a lateral branch on the left side of the next succeeding internode restores the function of producing another axial joint to the right side of the growing-point.

That there are exceptions to this rule has been shown above by the description of odd-numbered branchless internodes ; but it must be remembered that these cases are rare.

From what has already been said of the laws which regulate the growth of *Crisia*, it is obvious that a representation of a colony can easily be reconstructed from a formula of the character introduced by Smitt ; and no further justification is required for the use of such formulæ.

In some specimens of *C. denticulata* the average number of zoœcia in an internode may be higher than in the one described ; and the numbers 13, 15, 17, and even 19 are by no means uncommon. It may often be noticed that, although internodes consisting of any given number of zoœcia do not seem to be arranged in any definite order in the colony, an individual colony may be characterised by the frequent occurrence of internodes with that number of zoœcia. Thus if the dominant number, in any particular case, be 11—and this seems to me the most common case—variations in the number of zoœcia in the internodes of that colony will apparently take place about the number 11 as a mean ; so that, although internodes of 9 or 13 zoœcia are common, there may be none of so many as 15 zoœcia. But if the colony have many internodes of 15 zoœcia, for instance, then it will probably be found that some of the other internodes have 17 or 19.

If the growth of the branch be complete, so that no more axial joints are to be formed, the terminal internodes, and especially those which have produced ovicells, may have a larger number of zoœcia than the internodes of the rest of the colony ; and the number of zoœcia formed before the growing-point exhausts its activity does not appear to be regulated by the laws which govern those internodes which are not terminal. But even the terminal internodes normally produce no more than a single branch (cf. *C. aculeata* and *C. ramosa*), the cases mentioned on p. 149 being the only ones in which two branches were noted to come off from the same internode.

The articulations of the lateral branches of this species are

alone sufficient to distinguish *C. denticulata* from the other British forms. They are situated at a very low level on the zoœcia which bear them, and each "basis rami" (Smitt) appears to be wedged in between two consecutive zoœcia (fig. 3), instead of being, as in other species, distinctly apposed to the outer side of one zoœcium (figs. 4, 6, 11). The branches usually originate from  $z_3$ ,<sup>1</sup>  $z_4$ , or  $z_5$ ; less commonly from  $z_2$  or from  $z_6$ .

The joints, both of the zoarium and of the rootlets, of this species are in nearly all cases of a jet-black colour, as recognised by most of the previous writers.<sup>2</sup> The young joints are, as in other species, uncoloured; but the black colour is in almost all cases very speedily acquired. Smitt and Busk do not mention this as a specific character, no doubt because they have given wider limits to the species than are accepted by most writers.

The ovicell<sup>3</sup> in all species replaces an ordinary zoœcium, and in this particular species it is usually borne on a lateral branch, and in most cases is situated at some distance above a joint. In the instances given in the formula on p. 146 the ovicell replaces the 4th, the 6th, or the 10th zoœcium of an internode. I have never seen it lower than 4th nor higher than 13th. It is usually very near the end of a branch, and this feature is well shown in pl. iv, figs. 2 and 4, of Busk's British Museum Catalogue (Part III). In one of my cases, however, thirteen zoœcia occurred above the ovicell, and eleven below it, and very rarely a joint may be developed above it. If the ovicell-bearing internode develops a branch, that branch is very seldom given off from a position higher than the zoœcium which corresponds to the ovicell on the opposite side of the branch.

<sup>1</sup> I.e. from the third zoœcium of either right or left side: the side from which a branch comes off has no significance unless considered in relation to other characters.

<sup>2</sup> Cf. Fleming, J., 'Hist. Brit. An.,' p. 540; Johnston, A., 'British Zoophytes,' 2nd ed., p. 284; Hincks, T., 'Brit. Mar. Polyzoa,' p. 423; &c.

<sup>3</sup> See also p. 169.





method of branching is well developed, the internodes composing the sympode are usually made up of five zoœcia, and that, although the branching may, in other parts of the colony, take place from  $z_2$  (or rarely from  $z_3$  or  $z_4$ ), well-developed helicoid cymes are invariably composed of internodes in which the branching takes place from  $z_1$ .

These helicoid cymes do not, however, agree with the method of branching defined under that term in text-books of botany, in that the main axes of the parts of the sympode are by no means suppressed. This is obvious enough from the formula, in which the first internode on the left side forms the basal member of a helicoid cyme developed on the left side of the branch; but it is, at the same time, the basal member of a long axis, which develops new cymes alternately on opposite sides; and the same is true of the other constituents of the sympodes. Thus each of the branches indicated in the formula, with the exception of those which are quite near to the growing-points, is again the basal member of a helicoid cyme; and these cymes are consequently given off alternately on opposite sides, not only by the internodes of the main stem, but also by the internodes of its branches of the second, third, and other orders. The number of members of which these helicoid cymes are composed decreases fairly regularly in a centrifugal direction.

Each internode is typically provided with one branch, and at the same time is composed of an odd number of zoœcia, just as in *C. denticulata*. It is not uncommon, however, to find branchless internodes, whose position in the colony may be illustrated by the formula—

$$(5 + r_1) + (6) + (5 + r^r);$$

and, just as in *C. denticulata*, these branchless internodes nearly always consist of an even number of zoœcia, most commonly of four or six, less often of two or eight. Exceptions to this rule are somewhat less rare than in *C. denticulata*, which this species so closely resembles in its method of branching. The exceptions are more common at the base

of the colony than elsewhere. The branch is developed on the same side as the basal zoëcium of an internode, and the last zoëcium is usually somewhat produced. In very rare cases, of which the specimen represented in fig. 6 is an example, two branches may be developed from the same internode.

The articulations which bear the lateral branches are relatively short; even when the branch is developed from  $z_2$  or  $z_3$  the "basis rami" is never wedged in between two zoëcia, as in the last species; and the joint which bears the branch is nearer to the aperture of the zoëcium which has developed it than in *C. denticulata*.

The number of zoëcia is typically five or seven, the former number being especially characteristic of the members of a helicoid cyme. As in *C. denticulata*, the definiteness with which the colony grows is frequently indicated by the regular repetition of the same forms of internode in a branch. Thus the greater part of the main axis of the branch whose formula is given on p. 154 is composed of internodes of the type  $(7 + r_2)$ ; in the main axis of the branch given off by the second internode of that stem,  $(5 + r_1)$  alternates regularly with  $(7 + r)$  until the end of the axis is nearly reached; while in the next line but one will be seen the formula of a branch composed of units of the type  $(5 + r_1)$ . The regular repetition of internodes of the type  $(5 + r_1)$  in the formation of most of the helicoid cymes is a further illustration of the same thing. In many other cases, however, no such regularity of arrangement was noticed. In a colony found at Plymouth in August, the dominant number of zoëcia was seven, although internodes with five zoëcia were not uncommon. But, in correlation with this increase in the normal number of zoëcia, it was found that several internodes of nine zoëcia occurred, and two of eleven.

In the terminal internodes the number of zoëcia may be larger; in one case observed it was as high as twenty, no growing-point being left.

The ovicell most commonly replaces the second zoëcium of a lateral branch (fig. 6), and is consequently the basal member of its own (axial) side; in other cases, however, the ovicell may

replace the third zoëcium above a joint (and it is then abaxial), but it is very rarely found higher in the internode. A branch is never given off by an ovicell.

As the age of the ovicell increases, fresh zoëcia continue to be added above it up to a certain point. The old ovicell seems to be always surmounted by a considerable number of zoëcia; in the specimen shown in fig. 6 there are, in addition to two incompletely formed zoëcia—the last that this branch would have produced—ten zoëcia above the ovicell. It must be noted that in this and other similar cases all zoëcia which are further from the joint than the ovicell are described as being above the latter. The second zoëcium of the right side in fig. 6 may not, at first sight, appear to be in this position, although an examination of the lower end of the ovicell at once shows its real place in the series.

A joint is seldom developed above the ovicell, and the growing-point usually completely exhausts its power of developing fresh zoëcia after a certain period.

The joints of this species are pale-coloured, or more usually yellow. In old parts of the colony the joints may become very dark, or almost black; this is especially true of those parts which form the starting-point for the regeneration of fresh branches. The joints are probably never so dark as they are normally in *C. denticulata*.

Smitt, in his valuable paper on *Crisia*,<sup>1</sup> gives a series of formulæ illustrative of the branching, &c., of the forms of this genus, and many of these formulæ illustrate in a most instructive manner the tendency of some at least of the species of *Crisia* to develop even-numbered internodes without branches. In his explanation to No. 8 of this series Smitt expressly points out that, in Nos. 4—8, shorter branchless internodes may alternate with longer internodes which have developed branches. It is a noteworthy fact that the greater number of the branchless internodes shown in these formulæ have an even number of zoëcia, and that the number is odd in most of those internodes which have developed branches. This fact seems, however, to

<sup>1</sup> "Krit. Förteckn.," I, 'Öfvers. af K. Vet.-Akad. Förhandl.,' 1865.

have escaped Smitt's attention. It must further be pointed out that some of the exceptions to the rule which has been so much insisted on above are probably due to the fact that some of the formulæ refer to *C. aculeata*, as is admitted by Smitt in two of the cases.

A very interesting abnormality of *C. eburnea* is figured in Pl. XII, fig. 5. The internode in question was the penultimate internode of a branch of a thoroughly characteristic colony, in which no other abnormalities were detected. In addition to bearing two lateral branches in a very unusual position, at its upper end, this internode distinguished itself by producing three zoœcia arranged in a row along the middle of its front surface, giving it, when seen from this side, an appearance very much like an *Entalophora*, for instance. The back of this internode appeared normal, and it was not obvious that any of the three growing-points borne by the internode was constructed in such a manner that it would have reproduced the abnormality in the next following internodes.

#### *C. aculeata*. Fig. 4.

I believe this form, which is in many respects intermediate between *C. eburnea* and *C. ramosa*, and which was originally distinguished as a species by Hassall,<sup>1</sup> to be a perfectly good species. Nearly all recent authors have regarded it as a variety of *C. eburnea*; this view is taken, for instance, by Hincks,<sup>2</sup> Busk,<sup>3</sup> Smitt,<sup>4</sup> &c. Even Johnston,<sup>5</sup> although inserting it as a distinct species, adds that he cannot persuade himself that it is more than a variety of *C. eburnea*.

My belief in the specific distinctness of *C. aculeata* rests mainly on the characters of the ovicell; since a particular form of ovicell (shown in fig. 4) is invariably found on colonies of

<sup>1</sup> Hassall, A. H., 'Ann. and Mag. of Nat. Hist.,' vol. vi, 1841, p. 170.

<sup>2</sup> 'Brit. Mar. Polyzoa,' p. 421.

<sup>3</sup> 'Cat. of Marine Polyzoa in Brit. Museum,' part iii, p. 4.

<sup>4</sup> Loc. cit.

<sup>5</sup> 'British Zoophytes,' ed. 2, p. 285.





zoëcium by means of a basal piece which is quite similar to that of a normal branch.

The spines shown in fig. 4 have been artificially bent backwards; in their normal position they curve over the front of the branches.

The number of spines developed on a colony is extremely variable; in a few cases spines are altogether absent, and the species could then hardly be distinguished with certainty from *C. ramosa*, were it not for the presence of the characteristic ovicells. Although, in one or two cases observed, an internode had developed spines on all or nearly all its zoëcia, it is not usual to find more than one or two spines on a single internode, while a large proportion of the internodes of a colony do not develop any of these structures. The spines most commonly occur on the lower zoëcia of an internode, and are commonly in the position—

$$(n + s_1 + r_2) \\ \text{or } (n + s_1 + s_2 + r_3);$$

being found on the abaxial side if the internode is, as is often the case, the basal member of a branch.

The spines may, however, be developed in other positions; thus it often happens that the last structure developed at the apex of a branch, before the growing-point ceases to grow, is a spine,<sup>1</sup> which is situated on the axial side of the last zoëcium, and is consequently in the position of the terminal zoëcium of the branch to the right of the ovicell in fig. 4.

In the specimens (most of them from Plymouth or Roscoff) which have come under my notice the presence of a single spine on a colony has been quite sufficient to enable the species to be identified with certainty as *C. aculeata*. It is perfectly true that the lower parts of the zoarium may have an eburnea-like appearance; but the colony, if well grown, seems always to acquire the *aculeata* form of the zoëcia and internodes towards the ends of the branches.

I have in no case found an ovicell of the type shown in fig. 6

<sup>1</sup> Cf. *C. acuminata*, Busk, "Challenger" Rep., part 50, pl. iii, fig. 1.

(*C. eburnea*) on a colony which, from the presence of spines or from other characters, was found to belong to *C. aculeata*. I cannot admit that there is sufficient evidence to show that this form is merely a variety of *C. eburnea*. Both in the form of its zoëcia and in its method of branching it is totally unlike this form, although it is sometimes with difficulty distinguished from *C. ramosa*.

The branches of *C. aculeata* are usually slightly incurved, but not nearly to the same extent as in *C. eburnea*; and it does not possess the well-developed helicoid cymes of the latter species. Many of the internodes bear two branches, usually on opposite sides, but more rarely on the same side. In well-developed colonies the terminal internodes, and especially those which possess ovicells, are commonly provided with two branches.

The number of zoëcia in an internode is extremely variable; it is usually small in the lower internodes of a stem, such numbers as 1, 2, 3, and 4 being common in this position. The next parts of the stem, and the basal parts of the lateral branches given off by it often assume an *eburnea*-like appearance, the internodes consisting of 5 or 7 zoëcia. At the ends of well-developed branches the number usually becomes higher; a terminal internode with 22 zoëcia has been observed, although this number is higher than is usually the case. When the terminal internodes have many zoëcia they usually bear two branches; but if the number of zoëcia is still larger the number of branches may increase to as many as five.

The position of the branches is another very variable feature. In the lower parts of a colony the branching takes place commonly from  $z_1$ ; while higher up, although some of the branches still come off from  $z_1$ , others are given off quite as commonly from  $z_2$ , and in many of these cases the zoëcium below the branch, and on the same side, bears a spine. Branching may, however, take place from the higher zoëcia of an internode, as from  $z_3$  or  $z_4$ ; and when several branches come off from the same internode, those which are last formed have a very high position in the internode. The

most striking case observed illustrating this point had the formula—

$$(7 + Ov. + 11 + r_1 + r_4 + r_5 + 6r + r_{10} + x).$$

It cannot fail to be remarked that the character of the branching is much more variable in this species than in *C. eburnea*.

The joints are usually yellow; the articulations which bear the branches are usually short, and are then very similar to those of *C. eburnea*; in some cases, however, they acquire the form characteristic of *C. ramosa*.

Near the ends of the branches, where most of the zoœcia have polypides, the ends of the zoœcia are, in most cases, long free tubes, and are thus strikingly different from those of *C. eburnea*. The free portions of the zoœcia are either gradually bent forwards from the point where they leave the branch, or they may be bent forwards at a distinct angle from this point. The curvature of the zoœcia is, in either case, different from that of *C. eburnea*. The zoœcia are distinctly longer and more “loosely aggregated” than in that species; and the branches are usually of slenderer habit (as recognised by Johnston<sup>1</sup>).

The ovicell is nearly always higher in the internode than in *C. eburnea*. In the average of a considerable number of observed cases the ovicell was in the position of the 5th—6th member of the internode above the joint, and thus replaced  $z_3$  or  $z_4$ . In one case the ovicell replaced the 8th zoœcium, and in another it was the 3rd unit of the internode; in no case was it found lower.

The stem is not usually jointed above the ovicell; and fig. 4 is, consequently, a somewhat exceptional case. As in the preceding species, the ovicell is normally borne by a terminal internode; and a considerable number of zoœcia may be added above the ovicell.

<sup>1</sup> ‘Brit. Zoophytes,’ ed. 2, p. 286.



*C. ramosa*, n. sp. Fig. 11.

Some of the characteristics of this species are well exhibited by the formula<sup>1</sup>—

[illegible]

The branching is seen to be very similar to that of *C. aculeata*, but the tendency, already manifested by that species, to develop more than one branch from an internode, is here carried much further, so that a considerable proportion of the internodes have two branches each, while the terminal internodes, if the colony is well grown, will be found to have at least two each.

The rule relating to odd- and even-numbered internodes, so characteristic of *C. denticulata* and of *C. eburnea*, here breaks down altogether, as, indeed, was the case to a considerable extent in *C. aculeata*. Odd-numbered internodes are not much commoner than even-numbered ones, and either kind may produce one or more branches, or be altogether branchless. The first branch of an internode is, however—as in other species—nearly always developed on the side of the basal zoëcium, and the last zoëcium of an internode is very often

<sup>1</sup> It is obvious that, in the case of the ovicell-bearing internodes, some of the branches are given off above the ovicells. For the purposes of the formula, however, the ovicell is counted as an ordinary member of the internode. A branch is probably never developed from the ovicell itself.

situated on the side on which the last branch is developed, thus causing the position of the basal zoëcium of the next internode, and consequently of the first branch of that internode, to be on the opposite side.

Just as *C. eburnea* is, on the whole, characterised by branching from  $z_1$ , so this species may be said to branch normally from  $z_2$ , or to produce branches on the type  $(r_n + n+2r)$ , where  $n$  usually represents  $z_2$  or  $z_1$ .

The extent to which this must be taken as a general rule may be understood by the following analysis of the complete formula of a well-developed colony :

|                                                              | Number of Cases. |
|--------------------------------------------------------------|------------------|
| (1) Internodes with one branch, originating from $z_2$ . . . | 24               |
| (2) Branches arranged on the type $(r_2 + 4r)$ . . .         | 5                |
| (3) " " " $(r_1 + 3r)$ or $(r_3 + 5r)$ . . .                 | 5                |
| (4) " " " $(r_2 + 2r)$ . . .                                 | 1                |
| (5) " " " $(r_2 + 3r)$ . . .                                 | 5                |
| (6) " " " $(r_1 + 2r)$ . . .                                 | 1                |
| (7) " " " $(r_3 + 4r)$ . . .                                 | 2                |
| (8) One branch only, originating from $z_1$ . . .            | 2                |
| (9) " " " $z_3$ . . .                                        | 8                |
| (10) " " " $z_4$ . . .                                       | 7                |
| (11) " " " $z_5$ . . .                                       | 3                |

Total number of internodes which had developed branches 63

Only eight of the completely developed internodes were branchless. Thus in this particular colony, in which no ovicells were present, and in which no internode possessed more than two branches—

30 p. c. of the branching internodes bore two branches ;

38 p. c. " " " one branch, originating from  $z_2$ .

32 p. c. " " " " " from other zoëcia.

100 p. c.

Or, adding together Nos. 1 to 4, the cases in which the branches come off from  $z_2$ , or in which the second branch is two zoëcia higher than the first, we find that these cases amount to 55.5 per cent. of the total number of branching internodes, and this may be taken as a case which does not exaggerate this feature of the branching.

Since some of the internodes which bore branches were immature, and had not had time to develop more than one branch, the figures would have been slightly different if the growth of the colony had been complete.

The symmetrical character of the branching noticed in other species is also found in *C. ramosa*. Thus the branches originating from an internode whose formula was  $(14 + {}_3r + r_5)$  developed altogether five internodes from which new branches were given off; in two of these cases the branch was borne by  $z_3$ , in two more by  $z_5$ , and in the last case by  $z_4$ ; and other cases of the same kind may easily be found.

The symmetry of the branching comes out with special clearness in the case of some abnormalities, of which the formula given on p. 163 is an example. Two consecutive internodes of the main stem represented in the formula give off internodes whose formulæ are identical. One of these gives off, on its right side, a lateral branch consisting of a single long internode bearing an ovicell, and the other gives off a precisely similar branch on its left side. Both of these ovicells have the same deformity, having developed a constriction at a particular point near their upper end; and it will further be noticed that the symmetry extends, to some extent, to the branches given off by the internodes which bear these ovicells.

Fig. 12 represents an abnormal ovicell or zoëcium of a type found in more than one colony. The growing-point appears to have started with the intention of developing an ovicell, and then to have altered its original purpose, and to have developed the incipient ovicell into an abnormal zoëcium. This alteration of purpose may have been due to the failure of the young ovicell to develop the egg<sup>1</sup> which is normally found in the immature ovicell.

The point which immediately concerns us at present is that each of two consecutive internodes of the same stem of this particular colony developed lateral branches, one on each side of the stem, and that each of these lateral branches bore an ovicell of this peculiar "suppressed" form. The same colony

<sup>1</sup> 'Proc. Cambridge Philosoph. Soc.,' vol. vii, p. 48.

possessed two more of these suppressed ovicells, two ovicells showing other abnormalities, and several normal ovicells.

In another colony a normal ovicell, with a "suppressed" ovicell on a lateral branch on each side of it, was noticed.

In another case (fig. 13) a single internode bore no less than four ovicells, and the colony to which this belonged possessed, in different parts, four internodes, in each of which two ovicells had been developed. It may be noted that the occurrence of two ovicells, side by side, in the same internode, is described by d'Orbigny<sup>1</sup> in *C. patagonica*, apparently as a normal feature of the species.

These cases, and the general remarks which have been made with regard to the branching of various species of *Crisia*, show that the growth of the colony is even more definite in its character than would appear from a superficial examination, and that in each particular species the tendency to vary is subordinated to certain principles of growth, which give rise to the special symmetry which characterises the species.

The number of zoëcia which compose an internode is even more variable in *C. ramosa* than in any of the other species. Generally speaking, the number is smaller near the base of the colony, and larger near its periphery, although this rule is by no means absolute. The length of the internode depends mainly on the number of zoëcia it possesses. The longest which was measured was a terminal internode in which growth had ceased, and which consisted of 28 units, of which the 10th was an ovicell; its total length being slightly more than 7 millimetres. These long internodes usually show a well-marked double curve, like a much elongated S, just as was remarked in *C. denticulata*; and they commonly bear 3, 4, or even 5 branches.

The appearance of the internodes depends greatly on the condition of the zoëcia. Near the ends of the branches the zoëcia generally have very long tubular mouths, and, for the most part, contain a functional polypide. This is especially

<sup>1</sup> D'Orbigny, A., 'Voyage dans l'Amérique méridionale,' tome v, 4<sup>e</sup> partie, 1839 and 1846, p. 7, pl. i, fig. 1.



true of actively growing colonies found early in the year : at a later period, when growth is less energetic, the apertures may be much less prolonged, and in many cases they are not more prominent than in some specimens of *C. denticulata*, even in the case of those zoëcia which are not closed by a diaphragm. Lower in the stem the tubular mouths are, in most cases, lost ; the zoëcium is closed by an oblique diaphragm, and no polypide is present. The internode is then a flattened structure, in which the apertures of the zoëcia project even less than in *C. denticulata*.

In an interesting abnormality found in August, two prominent tubular apertures occurred, side by side; the extra zoëcium being in the position which would normally have been occupied by a basis rami.

Busk<sup>1</sup> has made rather a point of the fact that in *C. conferta* the free tubular portion of the zoëcium is not a mere production of the peristome, but presents "the same puncturation as is seen on the rest of the cell." This is certainly the case in *C. ramosa* and in *C. aculeata*, and to a less extent in *C. denticulata*. *C. eburnea* is, in fact, the only species I have examined in which the tubular portion is usually merely a thin prolongation of the peristome.

The average position of the ovicell is somewhat higher than in *C. aculeata*; but that it varies greatly in position is obvious from the formula—

$$(4+Ov.+19+r_1+_2r+_4r+r_6)+$$

$$|=(13+Ov.+9+_2r+r_3+_8r+r_9)+$$

It has never been noted to be lower than 4th in the internode; but it is seldom so low as this. The branch may be jointed above the ovicell, although most commonly the ovicell is borne by a terminal internode, which usually possesses at least two branches. When several branches are developed, two of them are usually developed not far above the ovicell, one on each side of the internode, whilst the other branches are developed from the lower parts of the internode. This is the case in

<sup>1</sup> 'Catalogue . . . Brit. Museum,' part iii, p. 7.

fig. 11, and in both the above-cited formulæ in which the ovicell replaces  $z_3$  and  $z_7$  respectively.

The joints of this species are yellow, or more rarely brown. They are never black. Rootlets are very freely developed from the base of the stem, and they may attain a great length. They usually originate rather low on the zoœcia and from their lateral edges. As in other species, they become very firmly attached to stones and other objects, and form creeping stolons, from which (as well as from rootlets which are not attached in this way) fresh stems may originate. The colonies do not so often consist of a single main stem as in *C. eburnea*. It is frequently remarked that the longest and most branched parts of the colony are lateral branches, and not parts of the main stems.

**Ovicells.**—In *C. ramosa* (figs. 10, 11) the ovicells are considerably larger than in any of the other species (see figures, all of which are drawn to the same scale, and table of measurements on p. 177). They are regularly pear-shaped, their main axis being straight; they are much inflated above, their curvature diminishing gradually in all directions from their most prominent portion. The aperture is in the form of a distinct funnel-shaped tube, which is considerably smaller at its base than at its mouth; and the mouth of the funnel, the actual aperture of the ovicell, is more or less circular. In the shape of the aperture this species differs from all the other British forms.

The tubular aperture is of course not present in incompletely developed ovicells: an account of the development of the ovicells will be given in a forthcoming paper. It must also be noted that the aperture is liable to be broken away in old ovicells, and that in many cases, where the ovicells or their contents are not normally developed, the tube itself is not formed. In normal and completely developed ovicells the shape of the aperture is, however, a perfectly characteristic and constant feature.

*C. aculeata* (fig. 4), which in some other respects is so similar to *C. ramosa*, has a much smaller ovicell than that

species (compare fig. 4 with fig. 11).<sup>1</sup> Its shape is very characteristic, its most prominent portion being considerably nearer its distal end than in *C. ramosa*. From this point the ovicell slopes off very suddenly towards its aperture, and more gradually towards its base, although this latter slope is steeper than in *C. ramosa*. The aperture is not borne on a distinct tube, but it lies in a characteristic position on the zoëcium next above the ovicell, on the same side of the internode. This zoëcium curves forwards round the back of the ovicell, the aperture of which is situated on it at the point where it makes its appearance above the ovicell.

In *C. eburnea* (fig. 6) the ovicell is large—considerably larger than in *C. aculeata*. Since the base of the internode which bears it is distinctly curved inwards, the ovicell itself has the same curvature at its base, as is best seen when the ovicell is looked at from the side. The ovicell is well inflated, and slopes away more gradually from its most prominent point than in *C. aculeata*. The aperture is quite characteristic; it is borne on a tube-like structure, distinctly broader at its base than at its free end, and instead of being circular, as in *C. ramosa*, it is transversely elongated, its lower border being often slightly convex towards the centre of the aperture.

In *C. denticulata* (fig. 3) the ovicell is fairly large, and usually becomes level with the flat surface of the internode near its base, the distal portion of the ovicell being very prominent. The aperture is not situated on a well-developed tube; it is not, however, on the surface of a zoëcium, as in *C. aculeata*, but is situated between two zoëcia, and it is very nearly sessile on the top of the ovicell, as was the case in *C. aculeata*.

In all four species the aperture is connected with the top of the ovicell at the point where the latter joins the front surface of the internode.

The importance of the form of the aperture appears to have

<sup>1</sup> It must, however, be pointed out that the ovicell of *C. ramosa* may be smaller, and that of *C. aculeata* larger, than in the particular specimens figured.

been almost completely overlooked by all previous writers on *Crisia*. The aperture is often said to be absent, as indeed it may be in injured or abnormal ovicells. As will be seen from a later communication, a normal ovicell is, according to my observations, never without an aperture from the time when the ovicell is first developed at the growing-point to the time when embryos are ready to escape from the ovicell. The calcareous aperture is, however, throughout the development closed by a thin cuticular membrane, and the presence of this membrane sometimes makes it difficult to see the aperture in those cases in which this structure is not borne on a distinct tube.

On breaking open an ovicell (fig. 10) it will be noticed that the aperture leads into a space partially separated from the rest of the ovicell by a valve-like structure of calcareous nature. This valve has very definite relations to the structures found in the interior of the ovicell, as will be described in my subsequent paper. It springs from the posterior wall of the ovicell, and passes obliquely forwards, being also attached to the lateral walls of the ovicell in such a way as to leave a more or less oval opening connecting the main cavity of the ovicell with the aperture of the latter. The valve is most developed at the back of the ovicell, and gradually dies away laterally as it passes to the front wall of the ovicell, where it no longer forms a distinct ridge.

This valve is developed in all the four species which are specially discussed in this paper, but it appears to be less well developed in *C. eburnea* than in the other species.

Note on *C. cornuta*, Linn., and *C. geniculata*, M.-Edw.

Until quite recently I had devoted no particular attention to these forms, the specific identity of which appeared to be perfectly established by such statements as those of Smitt,<sup>1</sup> to the effect that they may both occur as branches of the same stem. But, having recently found some ovicells of *C. geniculata*, I cannot help believing that the two forms are speci-

<sup>1</sup> 'Öfvers.,' &c., 1865, No. 2, p. 128.



fically distinct, and a more careful examination of *C. cornuta* has convinced me that Smitt's statement may be explained in such a way that it is unnecessary to follow him in his conclusion.

Even if the two forms are not really distinct, it appears to me worth while to call attention to what would then be an interesting case of a definite variation of the ovicells correlated with the presence or absence of spines on the zoëcia.

*C. geniculata* consists typically of a series of internodes, each of which is composed of a single zoëcium; from opposite sides of this zoëcium arise a pair of branches which are not quite at the same level. An excellent figure of this form is given in Busk's 'British Museum Catalogue' (part 3), pl. i, fig. 2. On comparing this figure with fig. 7 on the same plate (representing *C. cornuta*), it will be seen that *C. cornuta* exactly resembles *C. geniculata* as far as those zoëcia which bear two branches are concerned; but that, as Busk points out (p. 3), one of the branches is usually replaced by a jointed spine.

It is obvious that spines will be absent in *C. cornuta* if two branches are developed from each zoëcium. Further, the spines are very readily broken off, and a close examination is then sometimes necessary to discover the small basis with which the spine articulates. From one or other of these causes I have several times observed branches of normal colonies of *C. cornuta* having a close resemblance to *C. geniculata*; and this may be the explanation of Smitt's statement referred to above.

In every case in which I have observed the ovicells—although I must add that I have not obtained many ovicells of *C. geniculata*—I have noticed the following characteristic differences between the two forms; a reference to Busk's figs. 2 and 10 (l. c., pl. i) shows that the forms examined by Busk were similar to those which I have myself found. Busk's fig. 4 (*C. geniculata*) does not, however, quite agree with the specimens which I have examined.

The ovicell of *C. cornuta* (fig. 9) is the basal and only member of its own internode; it bears a lateral branch on each side, these branches originating at not exactly the same level.

After the branches have been given off, the ovicell becomes perfectly free, and is in this part considerably inflated. The tubular aperture arises near the back of the ovicell, and is usually bent somewhat backwards from its point of origin; so that, in looking at the branch from its "front" surface, the base of the tubular aperture is nearer to the observer than its distal end.

In *C. geniculata*, on the contrary, a common arrangement is as follows:—The basal member of the internode is an ordinary zoëcium (figs. 7 and 8), which gives rise to the ovicell as the second member of the internode. Immediately above the ovicell is another zoëcium, which gives off a lateral branch near the level of the upper end of the ovicell. The basal zoëcium itself gives off a branch on the opposite side to the ovicell. The internode may thus be represented as—

$$\begin{array}{c} (1 + Ov. + 1) \\ | \qquad | \\ 1' \qquad r_2 \end{array} \quad (\text{counting the ovicell as the basal member of its own side}).$$

The ovicell itself is distinctly smaller than in *C. cornuta*, and is not much inflated at its upper end. Its tubular aperture is most distinctly bent forwards from its base, sometimes at a very sharp angle, and the actual aperture is smaller than in *C. cornuta*. Moreover, the ovicell is not free, as in the latter species; the upper zoëcium of the internode being closely attached to its back along the greater part of its course. This zoëcium ultimately becomes free from the ovicell, and curves forwards above the upper end of the latter.

In other cases the ovicell may be the third member of the internode, each of the two zoëcia below it giving off a branch; and two zoëcia, each bearing a branch, may occur above the ovicell. The commonest arrangement seems to be either that given in the above formula, or the occurrence of three branch-bearing zoëcia, one of which is below the ovicell and the other two above it (cf. Busk's fig. 2). The ovicell is, in any case, not the lowest member of the internode, and one or two zoëcia are always attached to its back.

*C. geniculata* is a slenderer and more delicate form than

*C. cornuta*, its zoëcia being distinctly longer and thinner than in that species; and spines seem to be never developed. As already remarked, however, parts of the colonies of *C. cornuta* may be devoid of spines.

#### Breeding Period and Occurrence of Species.

The specimens from which the following statements are made have been received at various periods from February to the end of August. I have to offer my best thanks to those who have most kindly assisted me by supplying me with material; and especially to the staff of the Marine Biological Association, Prof. H. de Lacaze-Duthiers, and Mr. J. Sinel.

The dominant species at Plymouth is certainly *C. ramosa*; although, strangely enough, I have not been able to obtain this form from any other place, except from a bottle found in the Morphological Laboratory at Cambridge. The contents of this bottle came either from the Channel Islands or from Arran!

At Plymouth, *C. ramosa* is found commonly at depths from 4 to 30 fathoms. It is particularly fond of growing on stones, but is found on other objects—e. g. glass bottles, shells, red seaweeds, *Cellaria*, and sponges. When it grows in the last position it appears to be in danger of being killed by the sponge, which grows over its branches. The *Crisia* is, however, generally able to keep pace with the growth of the sponge, so that only the basal parts of its colonies are killed, or at least prevented from having functional polypides by the sponge. The specimens growing at 4—6 fathoms were usually much more luxuriantly branched than the few specimens which I received from 20—30 fathoms; and ovicells were obtained only from those growing under the first set of conditions.

*C. eburnea* is also common at Plymouth, but is almost always found on red seaweeds or on *Sertularia*. The restriction of various species of *Crisia* to particular seaweeds, &c., has been often noted by previous observers.

Winther<sup>1</sup> has stated that, in the Danish forms of this species,

<sup>1</sup> G. Winther, "Fortegnelse over de i Danmark hidtil fundne Hav-Bryozoer," 'Naturh. Tidsskrift' (Kjøbenhavn), 3 Raekke, vol. xi, 1877-8, p. 7.

the effects of the brackish water of the Baltic can be easily observed by comparing colonies found in different localities. Those which are nearest to the Baltic are said to have internodes consisting typically of three zoœcia; in those most exposed to the North Sea the internodes have seven zoœcia; and in colonies from intermediate localities they have five zoœcia. I have not been able to observe any definite correlation between the character of the colony and the conditions under which it was growing.

*C. aculeata* is less common at Plymouth than either of the preceding species; it was found on stones, red seaweeds, and sponges, usually from 4—5 fathoms.

*C. cornuta* was fairly common, mostly on red seaweeds; while *C. denticulata* was seldom found at Plymouth.

By the kindness of Prof. de Lacaze-Duthiers I received a large supply of *Crisia* from Roscoff in June. The species most common at Roscoff appear to be *C. aculeata*, *C. denticulata*, and *C. cornuta*. *C. geniculata* was less common, and only a few fragments of *C. eburnea* were found.

From Mr. J. Sinel I have received numerous specimens of *C. denticulata* and *C. cornuta* found at Jersey; and a smaller supply of *C. eburnea*, *C. geniculata*, and *C. aculeata*. The Jersey specimens of *C. denticulata* were found between tide-marks, while Smitt<sup>1</sup> states that *C. denticulata* is pre-eminently a deep-water form. It is, however, probable that Smitt's specimens did not really belong to this species.

I have also obtained specimens of several species from Guernsey and the Scilly Islands.

Smitt's valuable contributions to our knowledge of the Polyzoa have been devoted, so far as they concern the genus *Crisia*, to showing that the "species" which have been distinguished in this genus are in reality "forms" of a single species.

In one of his later papers<sup>2</sup> Smitt remarks, speaking of the forms of *Crisia* discovered in the expedition to which his paper

<sup>1</sup> 'Öfvers.,' &c., 1865, No. 2, p. 138.

<sup>2</sup> "Recensio, syst. . . . Bryozoorum . . . Novaja Semlja, &c.," 'Öfversigt. af-K. Vet.-Akad. Förhandlingar,' 1878, No. 3, p. 12.



refers, that he has united all these forms in a single species; and adds, "Auctores vero si sequi volumus, unamquamque fere coloniam speciem distinctam habebimus."

Although not in the least denying the difficulty of finding satisfactory specific characters other than those derived from the ovicells, the result of my investigation has been to convince me that Smitt has gone too far in denying the specific value of certain of the forms of *Crisia*. My results may possibly have to be explained by a suggestion which Smitt himself throws out, to the effect that although the series represented by the various forms of *Crisia* living in different localities is one in which practically none of the stages in the evolution of the species have been lost, "vivit multis in locis altera vel altera forma tam constans, ut species bene distincta facile censeatur."<sup>1</sup> Unfortunately, all the localities from which I have been able to obtain *Crisia* are comparatively close to one another; but I have found no essential differences between the forms of the same species from different localities.

Until it can be shown that any two or more of these forms can be developed as branches from the same stem, or as stems from the same rootlet, or at least that they can be produced as descendants of the same form, it appears to me that it will be impossible to deny to them the rank of species.

*C. eburnea* begins to breed at Plymouth as early as February; ovicells are present in great numbers during March, April, and May. Towards the end of the latter month they disappear, and are not normally present on colonies found in the summer. March and April appear to be the months when ovicells are most common.

*C. ramosa* commences to breed (at Plymouth) in April; a few of the colonies found at this period have young ovicells. In May young ovicells are very common; and the breeding period continues from this time until August at any rate. Immature ovicells may be found even at this time, but they are then becoming uncommon. The breeding season is probably at its height in May and June.

<sup>1</sup> 'Öfvers.,' &c., 1867, No. 6, p. 461.

*C. aculeata*.—A large proportion of the specimens found at Roscoff in June possessed numerous ovicells. At Plymouth ovicells were also found in April and May. The breeding season is probably much the same as in the last species.

*C. denticulata*.—The only specimens obtained in which ovicells were common were found in Guernsey and Jersey in the summer (June to August), which may probably be regarded as the period at which the breeding season is at its height.

*C. cornuta*.—The ovicells appear to be commonest in April and May.

*C. geniculata*.—The only specimens in which I have found ovicells were obtained in the summer (June to August). I have not found this species at Plymouth.

TABLE OF MEASUREMENTS (IN MILLIMETRES).

|     | C. denticulata.                                          |              | C. eburnea. |                                                    | C. aculeata. |             | C. ramosa.  |             |              |        |  |
|-----|----------------------------------------------------------|--------------|-------------|----------------------------------------------------|--------------|-------------|-------------|-------------|--------------|--------|--|
| A { | Length of long terminal internodes                       | 3.10         | 3.77        | 2.00                                               | 2.63         | 2.90        | 3.70        | 4.10        | 4.40         | 7.06   |  |
|     | Number of zoëcia . . .                                   | 15+Ov.       | 16+Ov.      | 10                                                 | 12+Ov.       | 13+Ov.      | 19          | 18+Ov.      | 19           | 27+Ov. |  |
|     | Average length of zoëcia                                 | .39          | .44         | .40                                                | .40          | .41         | .39         | .43         | .46          | .50    |  |
| B { | Length of long internodes (not terminal)                 | 2.80         | 2.87        | 1.83                                               | 2.26         | 1.80        | 2.10        | 2.23        | 5.90         | 6.46   |  |
|     | Number of zoëcia . . .                                   | 15           | 15          | 9                                                  | 11           | 10          | 10          | 10          | 20           | 23+Ov. |  |
|     | Average length of zoëcia                                 | .37          | .38         | .41                                                | .41          | .36         | .42         | .44         | .59          | .54    |  |
| C { | Length of axial internodes with average number of zoëcia | 1.87         | 2.13        | 1.43                                               | 1.00         | 1.13        | 1.20        |             | 2.43         | 3.43   |  |
|     | Number of zoëcia . . .                                   | 11           | 13          | 7                                                  | 5            | 5           | 6           |             | 9            | 11     |  |
|     | Average length of zoëcia                                 | .34          | .33         | .41                                                | .40          | .45         | .40         |             | .54          | .62    |  |
| D { | Distance from aperture to aperture                       | .27—         | .40         |                                                    | .30—         | .41         | .34—        | .40         | .38—         | .70    |  |
|     | Total length of zoëcium .                                | .64—         | .83         |                                                    | .54—         | .73         | .60—        | .80         | .83—         | 1.30   |  |
|     | Breadth of zoëcium . .                                   | Average .66  |             | Average .56                                        | Average .56  |             | Average .64 |             | Average 1.00 |        |  |
| E { | Breadth of zoëcium . .                                   | .08—         | .11         | .08—                                               | .10          | .07—        | .09         | .08—        | .11          |        |  |
|     | Breadth of branch . . .                                  | Average .09— | .10         | Average .09                                        | .09          | Average .08 |             | Average .09 |              |        |  |
|     | Length of "basis rami" .                                 | .22—         | .30         | .19—                                               | .24          | .16—        | .25         | .20—        | .33          | .56    |  |
| F { | Length of "basis rami" .                                 | .19—         | .24         | .18—                                               | .24          | .14—        | .35         | .30—        | .56          |        |  |
|     | Length of ovicell . . .                                  | .63—         | .80         | Average .22                                        | .22          | Average .19 |             | Average .38 |              |        |  |
|     | Length of ovicell . . .                                  | Average .73  |             | Average .67                                        | .40—         | .70         | .47—        | .67         | .70—         | 1.23   |  |
| G { | Length of ovicell . . .                                  |              |             | N.B.—The lower limit here given is unusually small |              |             | Average .63 |             | Average 1.00 |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| H { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| I { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
|     | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |
| J { | Length of ovicell . . .                                  |              |             |                                                    |              |             |             |             |              |        |  |

In **A**, **B**, and **C** of the above table the length of the internode is measured from joint to joint, or from joint to apex of branch if the internode is a terminal one. The "average length" of the zoëcia is obtained by multiplying the length of the internode by two, and dividing by the total number of zoëcia. It is obvious that this does not give the total length of the zoëcium, since the zoëcia overlap one another; but an approximation to the distance from mouth to mouth of the zoëcia is obtained. This gives a more accurate average than any single measurement of this distance would give, since the distance is variable in connection with the extent to which the tubular apertures of the zoëcia are developed, and with other circumstances. For the purposes of this calculation an ovicell is counted as an ordinary zoëcium. If the length of the internode of five zoëcia shown in fig. 6 (*C. eburnea*) be compared with that of a corresponding number of units, beginning at the base, of the ovicell-bearing internode in the same figure, it will be seen that the presence of the ovicell does not affect the result so much as would be expected at first sight, and the error due to counting the ovicell as a zoëcium is further lessened by the fact that this structure is nearly always borne on an internode which consists of many zoëcia.

In **D** the length is measured from any point of an aperture to the corresponding point of the aperture of the next zoëcium on the same side of the internode, and in making this measurement two zoëcia whose tubular mouths were about equally developed were always chosen.

**E** gives the total length of zoëcia with well-developed tubular apertures from the point where their cavity disappears at their proximal end to the furthest point of their apertures, the measurements being made from transparent (Canada balsam) specimens.

**G** is measured immediately above the aperture of a zoëcium.

**H** gives the length of the base with which a lateral branch articulates.

**I.** The length of the ovicell is estimated by drawing an imaginary line joining the point where the zoëcium next



below the ovicell on the same side becomes free from the internode (if any tubular aperture is developed), with the corresponding point on the zoëcium next above that zoëcium on the other side of the internode. The "length" of the ovicell is the distance of the middle point of the line drawn as above to the uppermost point of the ovicell, exclusive of its tubular aperture, if any.

This measurement, on the whole, gives the most constant results. The relation of the zoëcia below the ovicell to the ovicell itself is very variable, and it is impossible to take one of these zoëcia as a fixed point, a more definite result being obtained by taking two as described above. In *C. eburnea*, if the ovicell is the second member of an internode the imaginary line is drawn from the base of the tubular aperture of the first member of the internode in a direction parallel to that connecting the apertures of the upper pairs of zoëcia in the internode (a method which usually gives more satisfactory results than might be supposed from fig. 6).

The numbers given in the table do not profess to give the total limits within which a given part may vary, but the limits given will probably be found to include nearly all the variations which are observed in the ordinary forms of colonies.

In cases where it seemed to me possible to define the normal size of any part, I have put this down as the "average" size.

An examination of this table shows that *C. eburnea*, *C. aculeata*, and *C. ramosa* form an almost continuous series, as Smitt indeed has stated. *C. denticulata* can hardly be confused with any of the other forms, amongst which *C. eburnea* is the one that resembles it the most.

It will be seen that in every single measurement given *C. ramosa* is the largest of the four species, although in the size of the entire colony *C. denticulata* surpasses it. In many of the measurements, however, the upper limit of *C. aculeata* overlaps the lower limit of *C. ramosa*. As these are the two species which most closely resemble one another, and which are probably very closely allied to one another, it seems to me that it is not possible, in all cases, to

distinguish between a colony of *C. aculeata* without spines and a small variety of *C. ramosa* unless ovicells are present.

*C. ramosa* further appears to me to be much the most variable of all the species I have examined, while *C. eburnea*, and, next to this, *C. denticulata*, are the least variable. The greater variability of *C. ramosa* comes out perfectly clearly by subtracting the minimum from the maximum measurements given in the table, and comparing together the results thus obtained for the different species. If this be done for **D—J**, it will be found that, in every case except **F** (in which the limits are the same for *C. ramosa* and *C. denticulata*), the greater variability of *C. ramosa* comes out, and usually with striking distinctness.

It will be noticed that the largest variations are in the length of the zoœcia, and, correlated with this, in the size of the ovicells. But though the ovicells are thus by no means exempt from variation, their principal specific character (the shape of their aperture) is retained throughout without material alteration.

The fact that a colony of *C. eburnea*, on which some unusually small ovicells were present, has been taken into account in the table, makes these structures appear much more variable than they are in normal cases.

## EXPLANATION OF PLATE XII,

Illustrating Mr. Sidney F. Harmer's paper "On the British Species of *Crisia*."

(All the figures were drawn with a camera lucida under a Zeiss A objective, and were subsequently reduced  $2\frac{1}{2}$  diameters.)

FIG. 1.—*C. denticulata*.—Young internode, with growing-point, seen from the back (Canada balsam preparation).

FIG. 2.—*C. denticulata*.—Illustrating the relations of an even-numbered internode (pp. 147, 148).

FIG. 3.—*C. denticulata*.—Ovicell (p. 169, &c.).

FIG. 4.—*C. aculeata*.—Ovicell (p. 168, &c.).

FIG. 5.—*C. eburnea*.—Abnormality (p. 158).

FIG. 6.—*C. eburnea*.—Ovicell (p. 169, &c.). The internode which bears the ovicell has two branches, an unusual arrangement.

FIG. 7.—*C. geniculata*.—Internode with ovicell; back view (p. 172).

FIG. 8.—*C. geniculata*.—Another ovicell, seen from the front (p. 172). Greatest diameter of ovicell = .208 mm.

FIG. 9.—*C. cornuta*.—Ovicell (p. 171).

FIG. 10.—*C. ramosa*.—Ovicell broken open to show the valve (p. 170).

FIG. 11.—*C. ramosa*.—Branch with ovicell (pp. 163, 168, &c.).

FIG. 12.—*C. ramosa*.—"Suppressed" ovicell (p. 165).

FIG. 13.—*C. ramosa*.—Internode which has abnormally developed four ovicells (p. 166).





## The Later Larval Development of Amphioxus.

By

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With Plates XIII—XV.

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IN the account of the development of the atrial chamber of *Amphioxus* which appeared in this journal last August, under the joint names of Professor Lankester and myself, it was stated that we had not been able to trace the progressive steps in the history of the gill-slits, during their passage from the unilateral position which they hold in the larva, to the symmetrical condition which we find in the adult; nor the translation of the lateral mouth of the larva to the anterior median position of the mouth in the adult.

We added that we had taken steps to obtain the critical stages in the living condition during the summer of last year (1890). I accordingly went down to Messina, for the second time, in July, to undertake the study and to make drawings of the living larvæ—being enabled to do so by the continued kindness of Professor Lankester, to whom for this, as also for invaluable assistance and advice since received, I am deeply indebted.

According to Professor Lankester's instructions I spent the months of July and August at Faro, daily fishing in the Pantano, and concentrating my attention on larvæ of the particular stages which were required. To obtain a large number of individuals in the desired condition of transition, from the extreme asymmetry of larval life to the nearly symmetrical condition which is reached with scarcely appreciable increase of size, was no easy matter. By examining daily a great number of larvæ I succeeded in obtaining an ample series of the

transitional forms, which were carefully drawn and described as observed in the living condition. These observations form the substance of the present memoir. I may possibly find it desirable to add to this hereafter an account of some of the structures involved, as determined by means of sections. For this determination I have an ample supply of preserved material.

#### HABITS OF THE LARVÆ.

The conditions under which the material was obtained last year differed in a curious way from those of the preceding year. In 1889 the larvæ of *Amphioxus* were present in the lake at Faro in great numbers, especially during the months of July and August; while last year, during the same months, they were comparatively rare, and their place seemed to have been taken by an incredible host of *Doliola* of the first or larval generation.

Exactly in what way the presence of vast numbers of *Doliola* affected the larvæ of *Amphioxus* I was not able to make out, because the spawning of *Amphioxus* was prolific in the extreme. Possibly the breeding of *Amphioxus* as a whole commenced rather earlier last year, and the larvæ may have taken to the sand mainly before July. I found numerous *Doliola* with ova and gastrulæ of *Sagitta* in their pharynx, sometimes as many as nine ova in one *Doliolum*. This might lead to the death of the embryos in question, as it appears to cause the death of the *Doliolum*. I did not, however, find the ova of *Amphioxus* in this position, or possibly only in one or two doubtful cases. At all events, the scarcity of the larvæ of *Amphioxus* at this particular time of the year was evidently in correlation with the great predominance of *Doliolum*.

Professor Kleinenberg informed me of an analogous fact, namely, that *Salpæ* are sometimes very numerous in the harbour of Messina, to the exclusion of other small pelagic organisms.

When placed in glasses containing fairly clean water the healthy larvæ of *Amphioxus* are seen to be suspended, apparently motionless in the water, in a highly characteristic vertical position. The suspension is no doubt effected by the movement of the long cilia with which the epidermis is provided.

It will be remembered as a curious circumstance that the adult habitually assumes a similar vertical position in the sand.<sup>1</sup>

The time of life at which the larva seeks its home in the sand varies greatly. While, on the one hand, I have dredged all the stages described below pelagically, I have, on the other hand, found larvæ which had only reached the third stage of the later larval development (see below ; also Pl. XIII, fig. 4) in the sand.

#### RÉSUMÉ OF THE ENTIRE DEVELOPMENT OF AMPHIOXUS.

The various phases in the development of *Amphioxus* may be conveniently arranged in the following way :

I. THE PERIOD OF EMBRYONIC DEVELOPMENT comprising the first thirty-two hours. It commences with the segmentation of the ovum, and ends with the formation of the mouth and first gill-cleft. According to the habit of the embryo, this period may be subdivided into—

(a) The time—namely, the first eight hours—during which the rapidly developing embryo is confined within the vitelline membrane, the successive stages being marked by the progress of the segmentation and gastrulation, the commencement of the formation of the myocœlomic pouches, the differentiation of the medullary plate, and the formation of the neurenteric canal.

(b) The time which elapses between the emergence of the ciliated embryo from the vitelline membrane, and the appearance of the mouth, first gill-cleft, and anus—the stages being marked by the successive formation of myocœlomic or archenteric pouches to the number of fourteen pairs. The myotomes

<sup>1</sup> The accounts of *Amphioxus* given by Rathke (Königsberg, 1841) and J. Müller (Berlin, 1844) have given rise to the impression that the usual status quo of *Amphioxus* is, to be lying on its side on the sand. As stated above, this is not the case. If it were so, it might be supposed to offer a simple explanation of the one-sided character of the larva—not that I think it would, however. J. Müller, indeed, says that *Amphioxus* is fond of burying the caudal half of its trunk in the sand, as an occasional divertissement. The lying on one side, however, is what is occasionally done ; and that is the result, not so much of a subtle inherited tendency to assume that position of rest, as of a gross incapacity on the part of *Amphioxus* to maintain its equilibrium in any other way when out of the sand.

which are added after this period never communicate with the intestine (Hatschek).

II. THE PERIOD OF EARLY LARVAL DEVELOPMENT during which fresh gill-slits appear one after the other—i. e. metamERICALLY—slightly to the right side of the median line (subsequently passing well up to the right side), to the number of twelve to fifteen. Towards the close of this period the longitudinal metapleural folds appear, and the closure of the atrium commences behind by the fusion of the small subatrial ridges which are developed on the inner faces of the metapleura.

III. THE PERIOD OF LATER LARVAL DEVELOPMENT during which the second row of gill-slits is formed on the right side; the first or primary row of slits passes across to the left side, the mouth assumes an anterior median and vertical position, the præoral cirri appear, and the endostyle is developed from its pre-existing rudiment.

IV. THE ADOLESCENT PERIOD when the young *Amphioxus*, having now attained most of the essential features of the adult structure, has definitely ceased to lead a pelagic life, and has taken up its abode in the sand, where its further growth in size and maturity is accomplished.

Of the above four main periods in the development of *Amphioxus*, the first has been studied by Kowalewsky (2) and more thoroughly by Hatschek (3); the second has been treated by Professor Lankester and myself (8), and the third forms the subject of the present paper. The fourth entails the consideration of the adult anatomy, for which Professor Lankester's paper (5) may be consulted.

The only observations recorded as to the course of events in the third period were made by Kowalewsky (1), whose all too brief account was considered so extraordinary that Balfour<sup>1</sup> was "tempted to suppose that his observations were made on pathological specimens."

Kowalewsky confined himself wholly to the question of the origin of the second row of gill-slits, and with reference to that he said that six disc-like thickenings appear together on

<sup>1</sup> 'Comparative Embryology,' vol. ii, 1885.



the right side above the row of primary slits. The secondary thickenings then become perforated and increase in size, and as they do so the primary slits pass under the pharynx, and so to the left side.

In his figures he places the first secondary slit immediately over the second primary. This is not correct. He did not describe the formation of additional secondary slits, and he did not observe the closure of any of the primary slits.

He was right as to the number of secondary slits which first appear together, and as to their formation on the right side above the primary slits, with the consequent moving of the latter round to the left side.

My observations on the gill-slits, therefore, in the first place confirm, in the second place correct, and in the third place add to Kowalewsky's description. Over and above this, the formation of the velum, the origin of the buccal or præoral cirri, the fate of the club-shaped gland, and the development of the endostyle will be found fully described below.

In the following account the later larval development will, for convenience, be divided into eight stages. The description of the separate stages will be followed by a brief summary of the stages, and this by a further summary of the facts relating to the various organs; the paper will be concluded with a few general considerations.

#### Stage I.—Figs. 1, 2, 20, and 21.

This is the stage immediately succeeding the older of the two stages figured in the paper referred to above (8), and the primary gill-slits and metapleura present very much the characters there described. Thus there are fourteen primary slits (fig. 1), most of which open directly to the exterior; but the last three or four discharge into the atrial tube, which, while closed behind, is widely open in front, where the right metapleur is seen to overhang the anterior slits.

The new feature with which we have now to deal is the appearance of a continuous ridge in the right wall of the pharynx above the row of primary slits.

On this ridge are developed typically six oval thickenings or enlargements, alternating with the primary slits and appearing simultaneously. They consist of a fusion of the pharyngeal wall with the body-wall at six different points, the first point lying above and between the third and fourth primary slits. They are the forecast of the second row of slits. Between the ridge with its nodal thickenings and the row of primary slits a longitudinal blood-vessel is seen to hold its course; this becomes eventually the ventral or subintestinal vessel which lies beneath the endostyle (see Lankester, 5).

The first and last thickenings, as Kowalewsky also noticed, are usually rather smaller than the intervening ones.

Slight deviations from the above mode of procedure occur. One of these is shown in fig. 2, where only five secondary thickenings have appeared at once, the first of them occurring between the fourth and fifth primary slits, while the one which usually has the first place at this stage has been somewhat retarded in its development.

In other cases, in which there are also only five thickenings, it may be the sixth that is late in appearing.

In the larva represented in fig. 2 there were only twelve primary slits. As mentioned above, the number of primary unpaired slits which are formed in the larvæ varies from twelve to fifteen. The most usual number is perhaps fourteen, while fifteen is exceptional.

In front of the gill-slits is seen the club-shaped gland, closely apposed to the remarkable patch of modified hypoblastic epithelium which, in the paper by Professor Lankester and myself (8) describing the larva, was termed the glandular tract. The nature of this organ has been enigmatical up to the present time; but I may as well at once call it the "endostyle," since it certainly becomes that organ.

The opening of the club-shaped gland into the cavity of the mouth—i. e. the intra-buccal orifice—lies at this stage slightly dorsal, and at a later stage quite dorsal, to the endostyle. This position of the internal aperture of the gland is important, as will appear in the sequel.

On the left side (figs. 20, 21) the large lateral mouth is seen with its margin ciliated. A ciliated groove leads from the præoral pit to the antero-dorsal margin of the mouth. Anteriorly the mouth ends in a sharp point; but in fig. 21, on close inspection, a very slight protuberance can be seen in the edge of the mouth projecting into the ciliated groove. This minute protuberance is the first indication of the combined forward and transverse growth by which the mouth moves from a lateral to an anterior median position.

Just below the mouth in front is the external orifice of the club-shaped gland, and some way behind this (fig. 20) is seen a small circular piece of homogeneous tissue, which is really a differentiation in the mesoblast of the lower lip of the mouth, showing through the outer integument, and is, in fact, the first element of those peculiar cartilaginous structures which form the skeleton of the præoral cirri. In fig. 21 there are two such elements, and they go on increasing in number by additions at both ends.

Running below the mouth, and then bending up to assume a dorsal position, is a ciliated band composed of columnar hypoblastic cells, forming a slight ridge in the pharyngeal wall. It is continuous in front with the base of the lower arm of the endostyle. On the other side there is a similar band running from the upper arm of the endostyle, but it cannot be seen in surface views at this stage.

In these, and all drawings of the left side, the anterior aperture of the nerve-tube described by Hatschek (3 and 4) is shown very clearly.

#### Stage II.—Figs. 3, 22, 23, and 24.

The distinction of this stage lies in the perforation of the secondary thickenings. The second to the fifth inclusive of the thickenings become perforated, as a rule, before the first and sixth; and, again, the first is usually perforated rather sooner than the sixth. This order, however, is subject to frequent exceptions. In fig. 3, for instance, the sixth secondary slit is open, but the first is not.

The apertures of the secondary slits are at first extremely small, and appear as dark spots, with transmitted light, in which long cilia are to be seen working.

In fig. 3 it will be noticed that a seventh secondary thickening has been added behind, but it frequently does not appear before the next stage.

In the same figure there are fourteen primary slits, but the fourteenth is only indicated in side view by a median depression in the floor of the pharynx. This means that the slit is in process of closure, as I found by occasional ventral views, which were obtained through the struggling of the animal when placed between slide and cover-glass. In this way the larva frequently got on its back, and became fixed in that position by the slight pressure of the cover-glass.

The endostyle and club-shaped gland present the same features as in the preceding stage.

The metapleura are also in much the same condition; but it may be noticed that while in fig. 1 the right metapleur concurs with the left about the region of the ninth and tenth primary slits, in fig. 3 it does so in the region of the seventh primary slit, thus indicating that the closure of the atrium has advanced forwards.

A view of the left or oral aspect of the larva (figs. 22—24) shows that in this stage the anterior extremity of the mouth becomes no longer pointed. This is due to a continuation of that hunching up of the antero-dorsal margin of the mouth into the ciliated groove, of which we saw the first indication in Stage I. It is ultimately carried to such an extent as to entirely change the original shape of the mouth. Meanwhile the anterior portion of the mouth sinks inwards deeper and deeper towards the other side of the body, so that it has to be examined with a deeper focus than in Stage I.

The upper part of the oral hood commences to form simply by the continued growth downwards of the upper margin of the præ-oral pit and ciliated groove; while the lower part, in which alone the buccal cirri have their origin, arises independently beneath the under lip of the mouth. Figs. 22—24, besides showing the



gradual change in the shape and level of the anterior half of the mouth, show also the progressive increase in the number of the elements of the buccal cirri—which, as they grow in number, grow also in size, and soon becoming irregular in their contour, show signs of growing out into tentacle-like processes.

I append a list of half a dozen observations on different larvæ at this stage, to show the nature and extent of the variations in the condition of the secondary slits. In all cases the first slit or thickening was above and between the third and fourth primary slits.

|             | Number of secondary<br>slits or thickenings. |   |   | Number of<br>open slits. |   |   | Thickenings not<br>perforated. |  |  |
|-------------|----------------------------------------------|---|---|--------------------------|---|---|--------------------------------|--|--|
| (i) . . .   | 6                                            | . | . | Nos. 2 to 5              | . | . | Nos. 1 and 6                   |  |  |
| (ii) . . .  | 6                                            | . | . | „ 3 „ 6                  | . | . | „ 1 and 2                      |  |  |
| (iii) . . . | 6                                            | . | . | „ 1 „ 5                  | . | . | No. 6                          |  |  |
| (iv) . . .  | 7                                            | . | . | „ 2 „ 5                  | . | . | Nos. 1, 6, and 7               |  |  |
| (v) . . .   | 7                                            | . | . | „ 2 „ 6                  | . | . | „ 1 and 7                      |  |  |
| (vi) . . .  | 5                                            | . | . | „ 2 „ 5                  | . | . | No. 1                          |  |  |

In the second row of the above table the numbers are inclusive.

It is worth noting here that the first primary slit is distinctly smaller than the second and following slits. If reference be made to the figure of the larva with three gill-slits given in the paper previously quoted (8) it will be found that the first slit is larger if anything than those which follow it. At a certain stage its growth is arrested, and later still, in a most remarkable way, it not only becomes relatively but actually smaller in size.

### Stage III.—Figs. 4 and 25.

In a larva of this stage the closure of the atrium has extended forwards so as to leave only a small portion unclosed in front, so that in this and all subsequent stages the gill-slits are entirely seen through the transparent wall of the atrium.

In fig. 4 there are twelve primary slits; the usual number at this stage is, however, thirteen. The secondary slits are seven in number; the first is circular in outline, and has just opened; while the seventh is only present as a thickening not yet perforated, and is also circular. The other secondary slits

are more or less elliptical in shape ; but it will be noticed that the third and fourth, which are the largest, are shaped like a plano-convex lens, their tops being flattened.

The increase in size of the secondary slits is accompanied by, and in fact is evidence of, a transverse growth by which the primary slits are gradually taken round to the left side.

The hindermost primary slits are always bent under the ventral wall of the pharynx ; but in fig. 4 this bending under extends to the more anterior gill-slits, namely, up to and including the fourth primary slit. This should be compared with figs. 1, 2, and 3.

The first primary slit is now smaller than we have hitherto seen it. The twelfth is very small, but when seen in ventral view does not yet show the definite signs of closing which will be described later.

The anterior wall of the mouth (fig. 25) has now sunk in or bent round so far that it can be easily seen through the right body-wall at this stage (fig. 4). This part of the mouth becomes the right half of the oral sphincter (velum of Huxley and Hatschek). As the mouth sinks towards the right side in this way, so also does the præoral pit ; and the latter gradually becomes flattened out as the development of the oral hood proceeds, and eventually becomes simply a ciliated tract on the under side of the oral hood, known in the adult as the " Räderorgan," which has been identified by Hatschek with the præoral pit of the larva.

The club-shaped gland and endostyle are approximately in the same condition as in the preceding stages, but in some larvæ of this stage I detected signs of the backward growth of the endostyle, in that the club-shaped gland was frequently seen to overlap the posterior edge of the endostyle.

Of the six secondary slits which have been described above as appearing at the same time, the first, which nearly always lies between the third and fourth primary slits, does not eventually become the first secondary slit, but it becomes the second, a new one being formed in front of it. The latter, however, occasionally appears as soon as the others. Thus

when all the secondary slits<sup>1</sup> are established, the one that stands first in position is not the first formed, but arises rather later than the six slits which follow it. The usually late appearance of the first secondary slit is possibly correlated in some way with the complicated growths which are taking place in the anterior region, and is not of any special significance; while, on the other hand, the simultaneous appearance of the greater number of the secondary slits is of considerable importance, as will appear later.

To bring the complicated nature of the transformation which is being effected vividly before the mind, it may be noted that, while the mouth is moving forwards, the endostyle is growing backwards, and the primary gill-slits are crossing bodily over to the left side.

The oral aspect of a larva at this stage (fig. 25) presents no particularly new features, but merely an extension of the processes described in the preceding stage as being in operation; thus the anterior portion of the mouth is seen at a still deeper focus, the apparent length of the aperture of the mouth when seen from the side has decreased, and the buccal skeleton has further advanced in development.

The following table comprises a few observations chosen out from a number to show some of the variations that are met with in this stage:—

|     | Number of<br>secondary slits<br>or thickenings. | Number<br>of<br>open slits. | Thickenings<br>not<br>perforated. | Position of first secondary slit<br>or thickening. |
|-----|-------------------------------------------------|-----------------------------|-----------------------------------|----------------------------------------------------|
| i   | 6                                               | Nos. 1 to 6                 | —                                 | Between 3rd and 4th primary.                       |
| ii  | 6                                               | „ 1 „ 5                     | No. 6                             | „ „                                                |
| iii | 5                                               | „ 1 „ 4                     | „ 5                               | „ „                                                |
| iv  | 6                                               | „ 2 „ 6                     | „ 1                               | „ 2nd and 3rd primary.                             |
| v   | 7                                               | „ 2 „ 6                     | Nos. 1 and 7                      | „ „                                                |
| vi  | 9                                               | „ 2 „ 7 and 9               | „ 1 „ 8                           | „ „                                                |
| vii | 5                                               | „ 1 „ 5                     | —                                 | „ 4th and 5th primary.                             |

<sup>1</sup> The primary slits form, as has been said, to the maximum number of fifteen. The secondary slits never normally exceed the number of nine. By far the greater number of the gill-slits of an adult *Amphioxus* must, therefore, be distinguished by the name of tertiary slits. The reason of this will become clear as we proceed.

This table will be intelligible if one compares what has been said above with reference to the first secondary slit with the position of the latter as given in the fourth column. In No. 7 it will be noticed that the first two secondary slits were late in appearing. No. 6 in the above table was obviously aberrant, but is interesting as exhibiting a hastening of the development of the full number of secondary slits. It was also slightly abnormal, since, although the ninth slit was well open, the eighth was only present as a thickening.

In all cases the eventual first secondary slit arises above and between the second and third primary slits.

The trifling confusion which may attend this description is unavoidable. The straightforward course of events, unhampered with variations, will be given in the summary.

#### Stage IV.—Figs. 5, 6, and 26.

This is a well-marked stage, characterised by a general increase in size of the secondary slits, accompanied by the dipping downwards or bending inwards of the dorsal wall in the largest of them—namely, Nos. 3 to 5 inclusive. The fusion of the down-growth from the dorsal wall with a small up-growth from the ventral wall of the slit (which, however, does not occur in this stage) results, as is well known from the figures of Kowalewsky and others, in the formation of the so-called tongue-bar of the slit.

The bending under of the primary slits from the right to the left side has now proceeded much farther; and the first primary slit is very much reduced in size. In the next stage we shall see that it closes up and ultimately disappears without leaving a trace.

In fig. 5 the thirteenth primary slit is seen in course of closure—that is to say, in a ventral view it would present the appearance shown in the case of the twelfth slit in figs. 9 and 10. There are seven secondary slits, the first being between the second and third primary slits.

The atrium is now completely closed anteriorly.

The most striking and unforeseen characteristic of this



stage is that the patch of modified epithelium which we have already spoken of as the endostyle has definitely commenced to push its way past the club-shaped gland, so that the latter now lies upon instead of behind it. This can also be expressed by saying that the endostyle has begun to fall away from its previously oblique position, so as ultimately to assume a longitudinal horizontal and ventral position. The incipient change of position of the endostyle is not accompanied in this stage by any growth in its length. The movement in bulk of a patch of modified epithelium from one position to another is sufficiently remarkable. It is probably, however, effected by the same transverse growth which affects the primary gill-slits. A consideration of the figures will show that it would be quite possible for a growth of this kind to have the effect of dragging down the at first very oblique endostyle.

It is not so much the primary obliquity of the endostyle, as its primary anterior position in the region of the first myotome, which should be especially noted.

A view from the left side of a larva of this stage is given in fig. 6, the object being chiefly to show to what extent the primary slits have come round to this side. The first primary slit is hardly recognisable from this side, but can just be seen with a median focus. In this larva there were fourteen primary slits; but the more usual number for this stage is perhaps thirteen, and, moreover, the thirteenth is usually in course of closure.

The oral hood, both as to its dorsal and ventral halves, is now well marked, and the cartilaginoid elements of the buccal cirri, of which there are six, are growing out into distinct tentacles, which give a crenate margin to the lower half of the oral hood. The external orifice of the club-shaped gland is undoubtedly present, but is covered over by the buccal cirri.

The apparent length of the mouth is rather greater in fig. 6 than is usual at this stage, and in this respect the condition of the mouth represented in fig. 26 is much more typical; a glance at this drawing will make it possible to understand that, while the contour of the mouth undergoes a complete

alteration in form, the actual size of the mouth does not materially change; though in the adult, of course, the relative size of the mouth or velum is much less than in the larva.

The variations in the condition of the secondary slits at this stage are not sufficiently striking to make it necessary to tabulate them. Their number, as a rule, varies between seven and eight; and almost invariably the first secondary slit appears at this stage between the second and third primary slits (fig. 5).

#### Stage V.—Figs. 7—10 and 18.

There is a considerable difference between the condition of a larva at the beginning of this stage and at the end of it, as will be seen by comparing fig. 7 with fig. 10. The constant peculiarity for the stage is, that although the primary slits have not quite attained their final position on the left side, yet the tongue-bars have commenced to grow down from the dorsal borders of the slits, but do not meet the ventral borders during this stage. There is also a feature which is more obvious in the preceding stages, and which we see for the last time in the present stage. I refer to the fact that the long axis of the secondary slits is at this and the foregoing stages parallel to the long axis of the body, and at right angles to the long axis of the several primary slits. This is the case even in fig. 10, where, however, one would probably fail to see it at a glance. It is important in that it is characteristic of the earlier stages in this period of the development.

In fig. 7 a view is given of the right side of a larva which had just entered on this stage. There are eight secondary gill-slits, the first, as one would expect, being between the second and third primary slits. The tongue-bars, as we have already seen, are in course of formation, and that of the third secondary slit has actually fused with the ventral border of the slit. Unfortunately in the larva here figured (fig. 7) the endostyle is not typical for this stage, being in the condition described in Stage IV. A characteristic endostyle is, however, shown in fig. 18. It has become much more horizontal in

position, and has grown a long way past the club-shaped gland. The latter is still present with its large intra-buccal orifice, but when seen in the living animal it seems to present signs of disintegration, the constituent cells assuming a loosely aggregated and turgid appearance preparatory to dissolution. The intra-buccal orifice is apparently the most persistent part of the gland. In fact, before the end of this stage the club-shaped gland atrophies altogether. From observations which I made on numerous larvæ belonging to this stage, I am inclined to believe that the cells of the gland break away from each other and pass into the alimentary canal, where they are possibly absorbed.

The ciliated hyperpharyngeal band of the right side, which has been referred to above, can now be seen proceeding from the upper arm of the endostyle; while the corresponding band of the left side proceeds from the lower arm of the endostyle. These upper and lower portions become, at a later stage, respectively the right and left halves of the endostyle; and, indeed, the latter—namely, the lower half—when looked at from the right side, is found to lie at a deeper focus than the right or upper half.

One of the most curious events of this stage is the closure of the first primary slit, which occurs very shortly after, if not at the same time as, the atrophy of the club-shaped gland. In fig. 7, which is a drawing of a younger larva than those represented in figs. 8, 9, and 10, the first primary slit can just be seen in side view; on the other hand, in the larva of which a three-quarter ventral view is given in fig. 9 it could not have been distinguished at all in side view; but a ventral view showed it in the condition of a minute aperture with cilia working in it, surrounded by cells in such a state of aggregation as to present a coarsely granular and dark appearance, which, combined with the loss of a sharp outline to its wall, is the distinctive feature of a closing slit, and enables it to be placed in marked contrast to a newly formed slit, of which the wall is clear and refringent.

In fig. 10 there is the merest trace of the first primary slit

just in front of the one marked "second primary slit." In fig. 8 there are indications of fourteen primary slits; the twelfth is, however, very small, and the thirteenth and fourteenth are on the verge of closure.

In both fig. 9 and fig. 10 the twelfth primary slit is shown in course of closure.

Fig. 10 is quite an exceptional view of a larva from the ventral or ventro-lateral aspect, obtained by the larva getting fixed on its back between slide and cover-glass, as a result of its struggles to get free. Such a complete view as is here represented can only be got very rarely, and when it does happen it lasts but a very few minutes, as the larva either speedily rights itself or dies.

The shape of the primary and secondary slits has been already described. The tongue-bars of most of the latter have fused with the ventral borders of the slits, but not one has done so in the case of the primary slits. It should be noticed, also, how the row of primary slits curves round to the middle ventral line behind; the twelfth is nearly closed, the eleventh would close later on, while the tenth might or might not close later: judging from the shape and size of it, one may say that the balance of probability is in favour of its not closing. In some of the primary slits there is a small up-growth from the ventral border of the slit, pointing towards and subsequently destined to fuse with the down-growing tongue-bar. All the slits are of course seen through the transparent atrial wall. The endostyle is seen anteriorly between the primary and secondary slits, and the buccal cirri are also well shown.

The mouth of this stage is shown in fig. 8. The lower portion of the oral hood, carrying the buccal cirri, is now more or less continuous with the upper portion into which the cirri have not yet extended; but, at the point of junction, a ridge is formed under which the cartilaginoid tissue eventually forces its way (cf. figs. 12, 13). The upper and lower portions of the oral hood become respectively left and right.

Thus, while the right and left halves of the oral hood are



independent of one another in their origin, the elements of the buccal skeleton arise entirely unilaterally from a single differentiation of mesoblast, which grows at both ends, and is situated primarily in the right or lower half alone of the oral hood, and secondarily continues itself into the left or upper half.

The lower edge of the oral hood is prolonged anteriorly as a ridge, into which the buccal skeleton is subsequently continued.

With regard to the variations met with in this stage, it is only necessary to say that the number of secondary slits varies from seven to nine; and as to the primary slits, it is usual to find the twelfth, sometimes also the thirteenth, on the point of closing; but I have found an instance in which, although there were nine secondary slits and the club-shaped gland was beginning to atrophy, yet there were fifteen primary slits, all in a healthy condition, with the exception of the first, which was closing.

#### Stage VI.—Figs. 11, 12, and 19.

The constant characteristic for this stage is that the primary and secondary gill-slits are of equal size and similar shape for the most part; and, excluding the first two and the last two on each side, they are approximately as broad as they are long. Further, the tongue-bars in most of them have fused with the ventral borders of their respective slits.

The first primary slit has entirely gone, and it is usual to find the eleventh primary slit, together with the twelfth and sometimes also the thirteenth (figs. 11 and 12), in a state bordering on closure. All the slits of the left side are now, as a rule, entirely confined to that side throughout their whole extent (cf. fig. 8), with the exception of the ninth, which bends under ventrally. The first slit of the left side in this and all succeeding stages is the original second primary slit; and similarly the ninth slit of the left side is the original tenth primary, and so on for the other primary slits.

Owing to the atrophy of the first primary slit, the first slit of the right side—i. e. the first secondary slit—comes to lie

opposite to the boundary between the first and second slits of the left side—and this is its final position.

Except when the above-mentioned ninth slit is found in an undoubtedly rudimentary condition, it is impossible to predict whether it would eventually close or not. My observations show that in the majority of cases it does close, but that, on the other hand, it frequently does not. I have never found a less number of primary than of secondary slits, so that when nine of the latter are formed (of which instances have been given above) it is certain that nine of the primary slits will persist.

On comparing fig. 12 with fig. 11 it will be found that the gill-slits of the former are not quite so far advanced as those of the latter, while the buccal cirri in the former have reached a higher stage of development than those of the latter. This is a slight variation.

The junction of the originally independent upper and lower portions of the oral hood is very distinct.

The anterior or right wall of the oral sphincter or velum has now passed so thoroughly round to the right side that it cannot be inserted in a drawing of the left side. Four velar tentacles make their appearance during this stage; they are much clearer, however, in the next stage.

The left ciliated (hyperpharyngeal) band is seen to join the left or lower half of the endostyle; the right or upper half of the endostyle is not seen from this side naturally.

In all the drawings of larvæ belonging to previous stages, from the left side, there is shown a peculiar structure, described as a nephridium by Hatschek (4), and placed just below and to the left side of the notochord in the region between the præoral pit and the mouth.

It presented the appearance represented in the figures, but I could not certainly detect cilia in it, and in fact was unable to understand its import. It seems to possess a superficial resemblance to the head-kidney of Annelid larvæ (trocho-spheres), but I can form no opinion as to the reality of any such resemblance. Hatschek discovered that it opened at one

end into the cavity of the mouth. (See also 8, pl. xxx, figs. 2—4.)

It requires, and would probably repay, further study. From the present stage onwards I could not recognise this so-called nephridium in the larvæ. The structure marked "*x*" in the figures is probably part of the ciliated tract of the præoral pit, seen through the oral hood.

In fig. 19 a view of the endostyle is given. It does not differ markedly from its condition in Stage V, extending about the same distance backwards between the anterior secondary and primary slits, but the absence of the club-shaped gland (of which feeble remnants are still shown) has to be noticed.

The number of secondary slits in this stage varies again from seven to nine.

#### Stage VII.—Figs. 13, 13*a*, and 14.

The slits—primary and secondary—have now begun to elongate in a direction at right angles to the long axis of the body.

A view of the left side of a larva is given in fig. 13. The first slit on this side (i. e. the original second primary slit) is simple and remains simple in the adult, while all the others become doubled by the formation of the tongue-bars.

The ninth slit of this side is very small and lies at a rather deeper focus than the other slits, so that in this case it would in all probability close. Behind the ninth (i. e. the tenth primary) is seen a rudimentary indication of the eleventh primary slit.

A separate view of the velum with the four velar tentacles is given in fig. 13*a*. Fresh tentacles arise between the four primary ones at a much later stage, when the gill-slits are about three times as long as they are now, and the endostyle twice as long. The final number of velar tentacles is twelve.

The right wall of the velum is not quite opposite to the left wall even yet: it is slightly in front of the left wall, and the extent to which it is so corresponds to what has been hitherto spoken of as the apparent length of the aperture of the mouth.

On the right side (fig. 14) it is usual to find eight slits. The

ciliated tract of the præoral pit is now beginning to alter its shape—becoming constricted in the middle. This constriction is carried still further in the next stage (fig. 15), and, as is already known, the ciliated tract subsequently grows out into several lobes, which give its characteristic appearance to the Räder-organ.

The most important feature in fig. 14 is the endostyle, which has increased considerably in length, reaching to the fourth slit. Its double nature is very apparent; the left (lower) half lying, as before mentioned, at a much deeper focus than the right (upper) half, so that the former only is seen from the left side (fig. 13).

In the larva represented in fig. 14 there were eight slits on the left side, and the ninth was seen to be in course of closure in the mid-ventral line.

We are now approaching the period in the larval development in which there are an equal number of slits on both sides of the body, all those on the left side being primary and all those on the right side secondary.

After a long interval, during which the gill-slits increase in vertical height and the endostyle extends further backwards, fresh tertiary gill-slits form behind on each side in a manner characteristic of later growth, and continue to do so throughout life.

The stage, then, with an equal number—seven to nine pairs—of primary and secondary slits, and before the formation of any tertiary slits, may well be called “the Critical Stage.”<sup>1</sup>

As a general rule the critical stage is characterised by the presence of eight pairs of slits, namely, eight primary slits on the left side and eight secondary slits on the right.

#### Stage VIII.—Figs. 15—17.

During this stage the critical stage, as defined above, is firmly established, and in fact may be said to commence as soon

<sup>1</sup> The remarkable fact that the number of pairs of gill-slits in the critical stage agrees approximately with the full typical number of the craniate gill-clefts should not be lost sight of.



as the fate of the tenth primary slit has been decided. In fig. 16 the ninth slit has not perfectly assumed its position on the left side, and so its permanence cannot be considered as being beyond question, although its size and appearance would lead one to think that it would persist and not close.

The length or vertical height of the gill-slits has further increased, and has been taken as the distinguishing feature of this stage.

Fig. 17 represents the last (seventh) pair of slits of a larva, in which not only had the tenth primary slit closed, but the ninth was also on the point of closing. The seventh slit of the right side is seen with a surface focus, the ninth rudimentary primary slit is seen with a median focus, and the seventh slit of the left side, which is doubling in, is seen with a deep focus.

The mouth or velum (fig. 16) is now anterior and median in position; the right and left walls of it are quite opposite to one another, and thus the apparent length of the mouth in side view is now reduced to zero.

The oral hood and buccal cirri have not yet, however, attained their final condition.

The endostyle (fig. 16) has greatly increased in length, extending to the fifth gill-slit on this side.

The right side of another larva of this stage is shown in fig. 15, chiefly for the sake of the endostyle. This is the last stage at which its double origin can be recognised. The right half of the endostyle is now seen to lie at the side of, and not above, the left half.

The ciliated hyperpharyngeal band appears to die out behind the pharynx. On this side there are eight (secondary) slits: on the left side of this larva there were also eight (primary) slits, and no indication of any more, so that it is a typical example of a larva which has entered upon the critical period.

It is important to emphasise the fact that the critical period, in which the larva has usually eight pairs of slits, has a considerable duration, in the course of which the only changes

that take place are the completion of the oral hood and the increase in the size of the gill-slits and endostyle, but no fresh slits are formed.

The close of the critical period may, therefore, be taken as the close of the entire larval phase of development. Up to this point all the gill-slits of the left side are primary—that is to say, they appeared at first as a single series on the actual right side of the larva, and were pushed across to the left side by the developing secondary slits. We shall find reason to suppose that the actual right side of the larva is not precisely identical with the morphological right side.

### Summary of the Stages.

In the following summary it will be found desirable to give a few measurements, which, however, have only an average value, the unit employed being a hundredth of a millimetre. It is also intended, for the most part, to ignore the variations which have been detailed above, and to state only what appears to be the usual condition of things, as inferred from observations on numerous larvæ.

Stage I. Right Side.—Fourteen primary slits, none closing; six secondary thickenings above the primary slits; endostyle in front of club-shaped gland, and therefore in front of all the gill-slits; atrium widely open anteriorly.

Left Side.—Large lateral mouth; apparent length (in this case the actual length), 45 units; 1 to 2 elements of buccal skeleton.

Stage II. Right Side.—Fourteen primary slits, fourteenth closing; secondary thickenings perforated; endostyle unaltered; atrium somewhat less widely open anteriorly.

Left Side.—Lateral mouth beginning to bend round in front; apparent length, 36; 3 to 5 elements of buccal skeleton.

Stage III. Right Side.—Thirteen primary slits; secondary slits simple; endostyle unaltered; atrium slightly open anteriorly.

Left Side.—Mouth still further bent round in front; apparent length ranges from 28 to 12, average about 25; 5 to 6

elements of buccal skeleton commencing to grow out as tentacles or cirri.

Stage IV. Right Side.—Thirteen primary slits, all bending under the pharynx, first very small and thirteenth closing; seven secondary slits, the new one appearing in front; the larger secondary slits commencing to double in, i.e. to form tongue-bars; endostyle extending a short way beyond the club-shaped gland; length of endostyle, 24; atrium closed anteriorly.

Left Side.—Primary slits are now partly on this side; average apparent length of mouth, 17; oral hood containing in its lower half incipient cirri.

Stage V. Right Side.—Twelve primary slits just visible at base of pharynx, twelfth closing; the first primary slit undergoes atrophy; eight secondary slits with complete tongue-bars in the larger ones; endostyle extends a long way past the club-shaped gland; length of endostyle, 28; club-shaped gland undergoes atrophy.

Left Side.—Primary slits not fully arrived on this side yet; tongue-bars commencing in the primary slits; average apparent length of mouth, 11; junction of upper and lower portions of oral hood.

Stage VI. Right Side.—Eight secondary slits, length equal to the breadth, measuring about 11 units, both in a direction parallel to long axis of body and in a direction at right angles to it; length of endostyle, about 34.

Left Side.—Ten primary slits (i.e. Nos. 2 to 11 inclusive), the eleventh closing; tongue-bars complete in the larger slits, which are equal in size to the corresponding secondary slits; mouth same as in Stage V; buccal skeleton commencing to penetrate the upper portion of the oral hood.

Stage VII.—Slits on both sides commencing to elongate in a vertical direction; average length of the central slits, 14.

Right Side.—Eight slits; length of endostyle, 39.

Left Side.—Nine slits, ninth (i.e. tenth primary) usually closing; apparent length of mouth or velum, 4; four velar tentacles.

Stage VIII.—Average length of central slits, 21.

Right Side.—Eight slits; length of endostyle, 45. The two halves of the endostyle are now definitely right and left.

Left Side.—Eight slits; apparent length of velum, 0.

Of the above stages, the fifth is certainly the most eventful. With regard to the last stage, it is well to be reminded that it inaugurates the critical period; that at the commencement of it there is often a rudimentary or doubtful primary slit behind; and, finally, that the presence of eight pairs of slits is not a universal rule, as there are sometimes only seven and sometimes nine pairs, but never normally more than nine.

The central feature, perhaps, of the foregoing observations is the development of the endostyle; next in importance comes the atrophy of the club-shaped gland and the first primary gill-slit during Stage V, and of almost equal importance and novelty is the atrophy of from four to six of the hinder slits of the primary series.

It goes without saying that there is no hard and fast line between any two consecutive stages. The division into stages is entirely for convenience, all the processes of development and transformation being gradual.

## SUMMARY OF THE HISTORY OF THE INDIVIDUAL STRUCTURES.

### 1. The Gill-slits.

The primitive or ancestral left row of gill-slits has, by a process which will be discussed later, been made to assume a position on the right side of the larva, where slits form to the number usually of fourteen, but occasionally fifteen.

The actual perforation in the case of each slit occurs at first at the base of the pharynx, near the mid-ventral line, and then extends upwards on the right side; the hindermost slits, however, retain their ventral position all through.

The slits which thus appear at first in a single unpaired series are called the primary slits.

The right row of gill-slits, which originally corresponded to the primary slits before the latter had forsaken their primitive



situation on the left side, has suffered retardation of development. When these retarded slits do appear, they are called, arbitrarily, the secondary slits.

The secondary slits form to the number usually of eight (sometimes seven and sometimes nine), above and alternating with the primary slits on the right side, appearing long before the completion of the atrium in front. The first secondary slit always lies between the second and third primary slits, and usually arises somewhat later than the succeeding six secondary slits, which develop simultaneously. It will be shown later that the simultaneous appearance of the secondary slits is only due to a masking of the metameric mode of formation.

As the secondary slits grow in size the primary slits gradually pass round to the left side, and meanwhile certain of them commence to atrophy. It is especially noteworthy that after Stage I the first primary slit becomes progressively smaller and smaller, until by the end of Stage V it has disappeared altogether. The view will be supported below that the closing of the first primary slit has a profounder significance than the closing of the hinder slits, or at any rate a different meaning.

By the closing of some of the primary slits their number is reduced to the same figure as that of the secondary slits, whether it be seven, eight, or nine. The primary slits which atrophy are, therefore, the following :

- a.* When 7 primary slits persist—Nos. 1 and 9, 10, 11, 12, 13, 14 close.  
*β.* „ 8 „ „ „ 1 „ 10, 11, 12, 13, 14 „  
*γ.* „ 9 „ „ „ 1 „ 11, 12, 13, 14 „

It is during the passage of the primary slits from the right to the left side that the completion of the atrium is effected.

The long axis of the secondary slits is at first parallel to the long axis of the body, while at the same time that of the primary slits is at right angles to the long axis of the body. The axes are equalised as soon as the two series of slits are properly adjusted on their respective sides, and then the slits on each side commence to elongate in a direction at right angles to the long axis of the body.

The formation of the tongue-bars commences very early in the secondary slits, but not in the primary slits until they are well round to the left side. The first slit on each side remains simple, and does not form a tongue-bar.

As soon as all the primary slits that are going to close have done so, the larva enters upon the so-called critical period. At the end of this period fresh slits begin to form normally on both sides, and continue to do so throughout life; these are called the tertiary slits in order to distinguish them.

Thus the critical period may be defined as the period in which the slits are arranged regularly—an equal number of pairs—in two rows, right and left; but all those on the left side are primary (in the sense in which the term is here employed), and all those on the right side are secondary.

## 2. The Endostyle.

The forecast of the endostyle appears in the embryo at a very early stage, namely, shortly after the formation of the club-shaped gland in front of which it lies. It consists of a patch of modified columnar hypoblastic epithelium, bending obliquely backwards and folded forwards upon itself, and lying on the right side of the mouth-cavity in the region of the first myotome.

The upper arm of the endostyle is at first much shorter than the lower arm; the former becomes the right half of the adult endostyle, and the latter becomes the left half.

The endostyle retains its original shape and its anterior position until some time after the first appearance of the secondary slits.

At Stage IV it begins to fall away, as it were, from its oblique position—the evidence of this being found in the fact that in this stage it extends a short distance beyond the club-shaped gland, so that the latter, instead of lying behind it, lies upon it (fig. 5). Although the endostyle has thus grown backwards, it has done so in bulk—that is to say, it has bodily shifted its position without increasing in length.

In Stage V, in addition to a further change of position, it

has commenced to grow in length (fig. 18), and now extends between two or three of the anterior secondary and primary slits.

In Stage VII (fig. 14) it has greatly increased in length, and is quite horizontal and ventral in position; but the upper and lower (i.e. right and left) halves are still perfectly distinguishable.

In Stage VIII the two halves of the endostyle, which have hitherto been in the relation to one another of upper and lower—though the lower has been in the later stages at a deeper focus than the upper—come to lie alongside of one another, and so become definitely right and left respectively.

In fig. 16 the two halves can just be recognised in front for the last time.

From the anterior extremity of each half of the endostyle a ciliated ridge composed of the pharyngeal epithelium passes dorsalwards, and becomes on each side the hyperpharyngeal band.

The anterior portion of the ciliated bands, where they curve dorsalwards from the endostyle, is undoubtedly to be compared with the pericoronal ridges of Ascidians which pass from the front end of the endostyle round the mouth to the dorsal lamina.

The following table shows the proportionate average increase in the length of the endostyle, together with the concomitant growth in length or vertical height of the gill-slits during the critical period. The unit of measurement, as before is  $\frac{1}{100}$  millimetre.

|   | Length of central slits. |   |   |          | Length of endostyle. |   |   |           |
|---|--------------------------|---|---|----------|----------------------|---|---|-----------|
| 1 | .                        | . | . | 27 units | .                    | . | . | 52 units. |
| 2 | .                        | . | . | 32 „     | .                    | . | . | 56 „      |
| 3 | .                        | . | . | 35 „     | .                    | . | . | 66 „      |
| 4 | .                        | . | . | 41 „     | .                    | . | . | 76 „      |

The endostyle does not reach the posterior end of the pharynx before the close of the critical period.

### 3. The Club-shaped Gland.

The development of this gland was made out by Hatschek (3). To his description of it has been added the discovery of the

intra-buccal orifice (8), and in the present paper the account of its atrophy. It arises in an embryo with nine to ten pairs of myocœlomic pouches, as a ventral transverse fold of the alimentary canal at a point intermediate between the first and second myotomes.

The fold extends up to the right side on the one hand, and ventralwards round to the left side on the other. It then becomes separated throughout its whole length from the alimentary canal, and develops an opening to the exterior on the left side, just below the lower margin of the mouth. Its intra-buccal orifice does not appear until a rather later period.

In striking contrast to all the other organs which adjoin it, the club-shaped gland does not undergo any change of position during what has been elsewhere called the process of symmetrisation (cf. figs. 4, 5, and 18).

During Stage V the club-shaped gland commences to atrophy, and towards the end of that stage it disappears altogether, the intra-buccal orifice being the last to go.

Considering their close proximity to one another, it would be natural to suspect that intimate relations existed between the club-shaped gland and the endostyle.

I made a few observations which seemed to show that the endostylar epithelium, at least before it has assumed its ventral position, is extremely delicate and liable to disruption.

When a larva had been confined for a very short time between slide and cover-glass, the epithelium of the endostyle would commence to give off numerous small round homogeneous ciliated bodies, which passed into the alimentary canal, and were there apparently absorbed.

This happened sometimes so soon after the larva had been placed upon a slide that I did not recognise it at first as a pathological phenomenon, as of course it was.

The fact that the endostyle is such a highly specialised and delicate (while young) structure suggests the idea that possibly a collateral organ like the club-shaped gland (concerning the morphology of which see below) might function as a sort of



special outlet for any nauseous substances entering the mouth, which might otherwise act injuriously on the endostyle. But the histological structure of the cells and the physiological properties of the gland require further study before any safe conclusion can be arrived at as to its function. The cells of the gland are ciliated, and the external orifice is surrounded by cilia.

#### 4. The Mouth or Velum.

The mouth arises in an embryo of about thirty-two hours as a minute circular aperture on the left side in the region of the first myotome (see Hatschek, loc. cit.). It then grows rapidly in size, and becomes a large lateral lens-shaped orifice, more or less pointed at both ends. From Stage I onwards it begins to grow gradually round the anterior extremity of the alimentary canal, the change in position being accompanied by great alteration in shape, consisting chiefly in the gradual equalisation of its diameters, which are at first remarkably unequal, the longer diameter being four or five times the length of the shorter one. The bending round of the mouth commences by a curious hunching up of the antero-dorsal margin of the mouth into the ciliated groove which runs from the præoral pit to the dorsal edge of the mouth.

By this means the previously pointed anterior extremity of the mouth becomes lost, and this part of the mouth then presents a truncated appearance. Meanwhile the anterior half of the mouth begins to bend inwards towards the right side, and, in fact, it eventually becomes the right half of the velum. The gradual attainment by the mouth of an anterior median and vertical position is fully illustrated in the plates at the end of this paper. The mouth is relatively much smaller in the adult than in the larva, but not actually so.

The edge of the larval mouth is provided with two kinds of cilia, namely, (i) a set of evenly distributed small cilia, and (ii) at a rather deeper focus much longer cilia, which hang together in groups at equal intervals. At the anterior extremity of the mouth in the earlier stages there is a group of very long cilia,

seen with quite a surface focus; and the whole external surface of the lower wall of the mouth is beset with small cilia (figs. 21 and 23).

Four velar tentacles are present in Stage VII, and this number continues all through the critical period. Eventually fresh tentacles arise and make up the number which occurs in the adult, viz. twelve.

### 5. Oral Hood and Buccal Cirri.

The right and left (lower and upper) halves of the oral hood develop independently of one another, and are also entirely independent of the metapleural or atrial folds, as will be readily gathered from the figures. It will be seen that the oral hood is formed mainly after the completion of the atrium. Professor Lankester has suggested (5) that "the oral hood is the præoral portion of the epipleural folds." This, of course, is now seen not to be the case.

The oral hood develops concurrently with the change of position of the mouth.

The upper portion of the oral hood arises between Stage II and Stage III as a fold of the integument overhanging the præoral pit and ciliated groove. Beyond the latter it merges into the upper margin of the mouth. This can be seen in section by referring to the paper which has been already quoted several times (8, pl. xxxi, figs. 13 and 14*a*). As the fold becomes larger it extends to the posterior extremity of the mouth, where it is met by the lower fold. Anteriorly the upper fold of the oral hood is continued somewhat beyond the limit of the præoral pit, and ends as a small ridge on the left side of the head region, just below the level of the notochord.

The formation of the buccal cirri is coincident with the development of the lower portion of the oral hood. In the adult the right and left halves of the oral hood are equally provided with cirri. It is, therefore, a most curious fact that the buccal cirri take their prime origin in the lower or right half only of the oral hood, and, after reaching a considerable stage

of development while still confined to the lower portion, they then grow into the upper portion also.

Thus the oral hood itself has a double origin ; it may be said to arise from two independent *ébauches* (to employ a French term which has not, when used in this connection, a satisfactory English equivalent, "forecast" being the nearest approach), while the system of the buccal cirri arises from a single *ébauche*.

The formation of the cartilaginoid elements which form the skeletal or supporting tissue of the buccal cirri, and which, taken together, may be referred to as the buccal skeleton, commences in Stage I. In this stage we find first one and then two minute circular and homogeneous differentiations in the mesoblast of the lower lip of the mouth, which can be seen through the outer integument. They appear some distance behind the external orifice of the club-shaped gland, just below the mouth. They commence to form before the mouth has begun to turn round in front.

In the succeeding stages fresh elements are added at both ends—anterior and posterior—so that the central elements are always the oldest ; and they soon begin to get irregular in outline, indicating a tendency to send out processes. Eventually each piece gives rise to one process.

As the differentiation of the elements of the future buccal skeleton proceeds the superjacent integument becomes raised up into a fold, which is the lower or right fold of the oral hood. About Stage IV the above-mentioned processes, which grow out from the separate elements, attain such a size that the integumentary fold over them is thrown into a series of pleats (fig. 6, &c.). By an extension of this pleating the buccal cirri of the adult are formed.

From Stage V onwards the lower fold of the oral hood meets the upper fold, and the point of junction is very plainly marked by a ridge, under which the buccal skeleton penetrates into the upper fold (figs. 8, 12, 13, and 16).

The lower fold is continued anteriorly some distance beyond the buccal skeleton as a ridge. Eventually the cirri extend

along the whole length of this ridge, as they do also along the upper fold.

It has been stated above more than once that the lower fold of the oral hood in the larva becomes the right half of the oral hood in the adult; and this half is, as Professor Lankester has pointed out (5), continuous in front round the extreme point of the body with the dorsal fin.

A little reflection will show that this is the condition that might be expected from the development of the lower fold, as shown in the drawings accompanying this paper.

It is important to note that the buccal skeleton grows at each end only, and that fresh elements are not formed interstitially. In the adult the median cirri are smaller than the others; and one would at first naturally suppose that these were the youngest, and that this was the point at which fresh cirri would be formed; as a matter of fact, however, the small size of the median ventral cirri of the adult is deceptive, for they are the oldest cirri, and new ones are only added at the free extremities, right and left, of the buccal skeleton.

#### GENERAL CONSIDERATIONS.

It is my intention to confine myself mainly to the attempt to give some explanation (or at least suggest one) of the three most prominent features in the larva of *Amphioxus*, namely:

1. The asymmetry.
2. The endostyle.
3. The club-shaped gland.

#### The Asymmetry of the Larva.

With regard, then, first to the asymmetry of the larva, we must begin by assuming—and the assumption will be supported later on—that in the primitive type from which the Cephalochorda diverged the notochord did not extend to the anterior end of the body, but, on the contrary, that the forward extension of the notochord in *Amphioxus* is secondary and adaptive; and further, that the primitive position for the



mouth was dorsal, as it is in the Ascidian tadpole.<sup>1</sup> The last assumption may appear to be the most arbitrary, but it is justified by the close affinities which exist between *Amphioxus* and the Ascidians, and which will be referred to again. It may as well be stated at once that the view I am about to put forward depends essentially on this way of regarding the forward extension of the notochord; and the immediate reasons for supposing it to be secondary are—

i. That it does not extend to the front end of the body at its first origin (see Woodcut, fig. 2).

ii. That its forward extension is an obvious advantage to *Amphioxus* when burrowing in the sand, which is its constant occupation after a certain period of development.

iii. The analogy of the Ascidian tadpole.

It is clear, then, that as the notochord pushed itself forwards beyond the anterior limit of the nerve-tube, the mouth would be compelled to forsake its primitive dorsal position—indeed, it would be forced, as it were, to one side, just as the anterior opening of the nerve-tube, which is at first actually dorsal, is made to assume a lateral position through the development of the dorsal fin; or, again, as the anus is similarly displaced by the caudal fin. It is a singular coincidence that the anterior neural pore, the mouth, and the anus are all deflected to the left side.

The lateral position of the mouth is thus supposed to be due to—or to occur in correlation with—the forward extension of the notochord; and it is evident that the same process which caused the mouth to rotate from a dorsal position to its present position on the left side in the larva would also by implication cause structures which were originally on the left side, or ventral, to pass across to the right side. There can hardly be any doubt that a semi-rotation of the ancestral pharynx has virtually taken place; the only question is, whether we are right in correlating it with the forward extension of the notochord. Supposing the ancestral mouth to have been small and circular, as it is in the Ascidian tadpole and in the very young larva of *Amphioxus*, it is possible that this

<sup>1</sup> See Balfour, 'Comparative Embryology,' vol. ii.

rotation (which, as above indicated, need only be a virtual phenomenon) may only have affected at first the most anterior portion of the pharynx, namely, the portion containing the endostyle, club-shaped gland, and first gill-slit.

In the ventral view shown in fig. 10 it will be noticed how the primary slits curve round posteriorly to the ventral middle line. It may be that formerly this tendency of the hinder slits to lie in the direction of the left side, though now only reaching the middle line, was more accentuated, and extended to the more anterior slits. The position of the primary slits which succeed the first on the right side has thus to be accounted for, and this is done by assigning the cause of it to the further adaptation which led to the huge size of the lateral mouth.

Having got on to the left side in the way above described, it was found advantageous to have as large a mouth as circumstances would permit, and accordingly the capacious mouth of the larva was established by the action of natural selection. A glance at the figures should carry with it the conviction that where the mouth is, there the gill-slits could not be.

Consequently the gill-slits which succeed the first develop, like it, on the right side, though, as we have seen, behind the region of the mouth they continue to retain a ventral position.

The lateral position of the mouth, then, is correlated with the forward extension of the notochord, and the unilateral position of the primary gill-slits with the lateral position of the mouth.

The rotation of the original left side gill-slits on to the right side has led, as might have been expected, to the temporary obliteration of the slits which properly belong to the right side, but it is not so much an obliteration as a retardation in development that has been effected. Thus, in a larva with apparently only a single series of gill-slits, there is potentially a double series; but, owing to the force of conditions which have been secondarily induced, one of the two rows is somewhat late in putting itself in evidence.

That we have to do here with a retardation in the development of a whole series of gill-slits is certainly proved by the fact that when the secondary slits do appear, they form mainly

not one after the other, as is usual with metamerised organs, but simultaneously. The successive or metameric mode of formation, in fact, obtains theoretically with the secondary slits of the larva of *Amphioxus*, only in this case it is latent, with the natural result that the slits appear simultaneously. The usually but not invariably late appearance of the first secondary slit is due to a further slight retardation, and is probably of no great significance.

The morphological mid-ventral line of the larva is indicated by the blood-vessel which lies dorsal to the primary gill-slits (fig. 1, &c.).

While the lateral mouth is serviceable to the pelagic larva, it would plainly be awkward to the animal during its career as a dweller in the sand—hence the necessity for the transformation which has been described above.

I have now given a possible explanation of the asymmetry of the larva of *Amphioxus*, namely, that it can be traced ultimately to the adaptive forward extension of the notochord. It is thus a purely ontogenetic phenomenon, and is not an ancestral character. It is only a temporarily superinduced condition, and is destined to give place after a brief existence to the inherent but latent bilateral symmetry of the organism.

#### AMPHIOXUS AND THE ASCIDIANS.

To pave the way for considering the significance of the endostyle and club-shaped gland of the larva of *Amphioxus*, we must institute a comparison between the embryo and larva of an Ascidian and of *Amphioxus*. The remarkable researches of Professors Ed. van Beneden and Charles Julin on the morphology of the Tunicates (6) have rendered it possible to do this the more effectually.

The peculiarities of an Ascidian embryo as described by the above-named authors are as follows (see Woodcut, fig. 1):

The enteric cavity at first of two portions, viz. a præchordal and a subchordal portion; the greater part of the lumen of the latter portion disappears at a very early period—it has been identified with the post-anal gut of higher Vertebrates

(see Balfour, 'Comp. Emb.,' vol. ii), into which the neurenteric canal opens; but there is every probability that there is no more post-anal gut in the Ascidian embryo than there is in the embryo of *Amphioxus*.

The mesoblast consists of two longitudinal bands differentiated from the primitive hypoblast.

These bands were formerly thought to be quite solid, but van Beneden discovered (*loc. cit.*) that anteriorly they occur as a pair of archenteric pouches (see Woodcut, fig. 1). The mesoblastic bands in the region of the so-called tail are solid, and consist of only a single layer of cells continuous ventrally with the remains of the hypoblast; but in front they consist of several layers of cells, which at first surround a short lumen which communicates, as stated above, with the enteric cavity on each side.

This single anterior pair of openings into the alimentary canal is the only indication in the Ascidians of archenteric pouches, and it is regarded by the authors as equivalent to the first pair of somites of *Amphioxus*. The rest of the mesoblast in Ascidians is unsegmented, and the general absence of mesoblastic pouches, with the exception of the first pair, is connected with the early atrophy of the posterior or subchordal portion of the alimentary canal, and is therefore probably a product of degeneration, and not an ancestral character.

In the embryo of *Amphioxus*, at a roughly corresponding stage (Woodcut, fig. 2) the notochord does not reach the anterior end of the body, but even at this early period it extends beyond the anterior opening of the nerve-tube (*cf.* Woodcut, fig. 1). Partly in front of the notochord and partly below it is a portion of the alimentary canal which lies in front of all the mesoblastic somites. This anterior chamber consists of a median portion and two lateral horns. The latter become constricted off from the chamber, and form the anterior intestinal diverticula, whose entire development has been fully described by Hatschek (3); while the median portion of the chamber simply merges with the anterior extremity of the alimentary canal, and ceases to be recognisable as a distinct chamber. As is well known, the left anterior diverticulum opens to the exterior



as the præoral pit, while the right one becomes the expanded head-cavity of the proboscis-like end of the body.

The anterior chamber with its two lateral horns, together

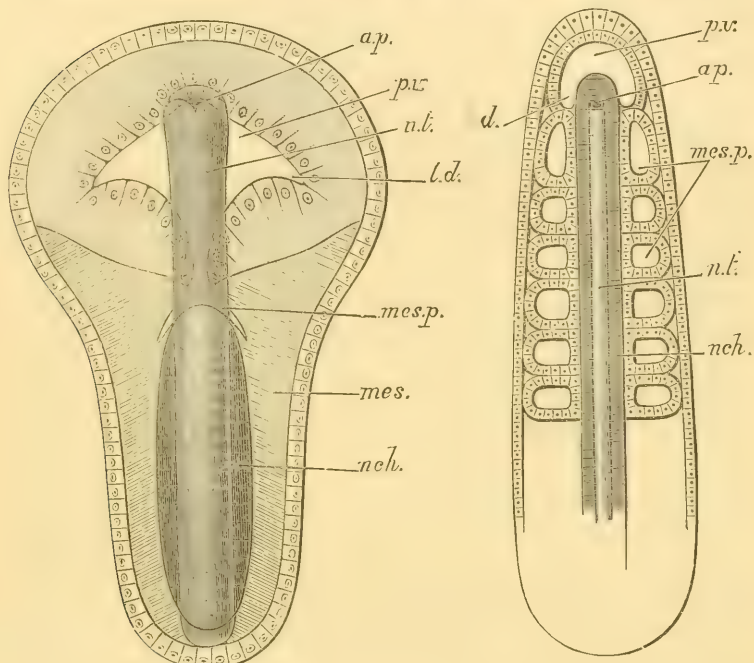


FIG. 1.

FIG. 2.

EXPLANATION OF THE WOODCUTS.

FIG. 1.—Diagrammatic dorsal view of an embryo of *Clavelina Rissoana* (modified after van Beneden and Julin). The mesoblast is represented in an extremely diagrammatic way. It consists really of large polyhedral cells.

*a. p.* Anterior neural pore. *n. t.* Nerve-tube, lying on the hypoblast in front and on the notochord behind. *p. v.* Præchordal vesicle. *l. d.* Lateral diverticulum (paired) of præchordal vesicle. *mes. p.* Mesoblastic or archenteric pouch: only one pair, equivalent to the first pair of pouches in *Amphioxus*. *mes.* Mesoblast. *nch.* Notochord.

FIG. 2.—Dorsal view of an embryo of *Amphioxus* of a corresponding age (after Hatschek).

Letters as above. The nerve-tube lies entirely on the notochord. There are numerous pairs of mesoblastic pouches.

with the region of the first myotome, is held by van Beneden and Julin to be homologous with the præchordal portion of the alimentary canal in the Ascidian tadpole. In the latter case, also, two horn-like outgrowths are developed from this part of the enteric cavity. They are subsequently met by two invaginations—right and left—of the epidermis, with which they fuse to form the so-called primary branchial canals. They become the right and left peribranchial cavities, and consist partly of epiblast and partly of hypoblast, and are considered to be homologous with the right and left anterior intestinal diverticula of *Amphioxus*, in which, however, the epiblastic element is wanting.

The peribranchial cavities then expand, and, growing round dorsally, fuse together to form the atrium. Meanwhile the characteristic gill-slits or stigmata have commenced to form, leading from the branchial to the peribranchial cavity, and finally the left portion of the latter cavity alone communicates to the exterior by the atriopore, the orifice of invagination of the right portion of the cavity having been lost. It is stated that probably the original communication of the primary branchial canals with the enteric cavity is lost, and that all the stigmata proper are secondary.

Thus the visceral wall of the Ascidian atrium is derived mainly from the hypoblast, while the peripheral wall is formed from the epiblast, and therefore the part of the atrium which leads to the exterior on the left side by means of the atriopore consists of epiblast; so that the præoral pit of *Amphioxus*, which is composed entirely of hypoblast, does not exactly correspond to the atrium of Ascidians, but is represented in the latter group by the visceral wall of the atrium, while the actual opening to the exterior of the præoral pit corresponds to the junction of the hypoblastic and epiblastic elements in the Ascidian atrium.

Neglecting their subsequent modifications, and supposing the epiblastic involutions to have been suppressed or reduced to zero in *Amphioxus*, the anterior intestinal diverticula of the embryo of the latter do actually coincide in every essential

respect with the primary paired atrial cavities, or, as they have been called, branchial canals of the Ascidian embryo; and the final opening to the exterior in both cases is connected with the original left diverticulum.

The two embryos represented in the woodcuts are thus regarded as practically identical with one another in all morphological respects. There is, in fact, no positive difference between them except in the relative proportion of parts of the body; the general absence of mesoblastic somites in the Ascidian being, as before stated, secondary and correlated with the atrophy of the subchordal portion of the archenteron.

It will possibly have been noticed that the forward extension of the notochord in *Amphioxus* commences at a remarkably early stage, months before the larva takes to the sand. This can be accounted for as a hastening of the development, a familiar phenomenon.

The divergence, then, between the two embryonic types consists in the great development of the præchordal region in the Ascidian embryo, and in the reduction of the same region in the embryo of *Amphioxus*.

It will have been inferred from what has been said that the tail of the Ascidian tadpole is regarded as the equivalent of the trunk of *Amphioxus*.

In the Ascidian the expanded portion of the præchordal region after giving rise to the peribranchial diverticula, becomes the branchial sac or pharynx, the floor of which is specialised as a glandular organ known as the endostyle.

The narrow portion of the præchordal vesicle, which also extends a very short distance beneath the anterior extremity of the notochord, gives rise to the œsophagus and stomach, while the intestine arises as a lateral outgrowth from the latter; but the extraordinary feature of it is that the intestine grows out from the stomach to the right of the mid-ventral line, and then passes across to open into the cloacal vesicle on the left side.

There can be little doubt that the ancestor of the Ascidians,

before the atrophy of the subchordal portion of the intestine, had an anus at the posterior extremity, as is the case with *Amphioxus*. This primitive outlet has, therefore, been supplanted by the development of the outgrowth from the stomach mentioned above, which van Beneden and Julin call a collateral diverticulum, and at the same time make the suggestion, which my observations tend to support, that the intestine of the Ascidian, arising as above described, is homologous with the club-shaped gland of *Amphioxus*.

#### THE ENDOSTYLE AND CLUB-SHAPED GLAND OF AMPHIOXUS.

We must now define the position of the endostyle in the larva of *Amphioxus* in order to grasp the situation of the club-shaped gland.

It has been already implied that in all probability the ancestor of *Amphioxus* was provided with a præchordal region and was symmetrical; and, in fact, resembled an Ascidian tadpole before the atrophy of the subchordal portion of the intestine.

In the larva of *Amphioxus* the endostyle lies at the extreme front end of the alimentary canal, which, as we have said above, represents the median portion of the anterior or 'præchordal' chamber, the lateral portions of which formed the anterior intestinal diverticula. It is further placed in the region of the first myotome; its peculiar oblique and lateral position has been already dwelt upon and explained. It also lies immediately in front of the club-shaped gland.

It results, first, from the analogy of the Ascidian tadpole, and secondly, from the position of the endostyle in the larva of *Amphioxus*, that it (the endostyle) was originally differentiated as a mid-ventral groove in the præchordal region. This primitive anterior position for the endostyle had been previously surmised by van Beneden and Julin, so that my account of its development in *Amphioxus* harmonises well with their conclusions.

The transverse growth which affected the anterior region



of the alimentary canal of *Amphioxus* at the time of the forward extension of the notochord naturally rotated the originally median ventral endostyle on to the right side in company with the collateral organ, now known as the club-shaped gland.

A comparison of the figures accompanying this paper, illustrating the development of the endostyle, shows clearly and beyond question that everything dorsal to the endostyle is on the right side of the morphological middle line, while everything below it is on the morphological left side. Thus the intra-buccal orifice of the club-shaped gland is on the right side, and the first and succeeding primary slits are on the left side—morphologically. At this juncture I want especially to emphasise the relative positions of the intra-buccal orifice of the gland and of the first primary slit.

The œsophagus and stomach of the Ascidian, which arise, ontogenetically, from the præchordal vesicle, are practically unrepresented in *Amphioxus*—the part of the vesicle from which they arise being suppressed. In fact, in their respective capacities as œsophagus and stomach they are not even theoretically present. Bearing this in mind, we can now proceed to compare the position of the club-shaped gland and its apertures with the position of the intestine in the young Ascidian.

In the larva of *Amphioxus* the club-shaped gland is situated behind the endostyle, i.e. at the posterior extremity of the primitive præchordal vesicle—in this respect agreeing with the intestine of an Ascidian. In both cases the aperture of the collateral diverticulum (whether gland or intestine) into the alimentary canal is immediately to the right of the mid-ventral line—in the one case actually, and in the other case morphologically; and in both cases the external aperture is on the left side. The point of origin of the club-shaped gland between the first and second myotomes also corresponds more or less closely with that of the Ascidian intestine. There is, therefore, a fairly strong case in favour of the homology of the two structures. The question still remains as to whether the club-

shaped gland is a rudimentary structure which formerly served as an intestine, or whether it is what van Beneden and Julin would call a primitive collateral organ, which in the Ascidians has come to function as an intestine. A great many considerations render it only reasonable to suppose that the intestine and anus of *Amphioxus* are primitive, and hence the club-shaped gland must be regarded as an ancestral organ occurring in company with a posterior anus.

Assuming, then, that the ancestor of *Amphioxus* possessed a collateral organ of unknown function situated behind the primitive median endostyle, with internal and external orifices as described, it is difficult to conceive how it can have become evolved, except by the modification of some pre-existing organ. The spectacle of an unpaired hypoblastic gland opening to the exterior on the one hand, and into the alimentary canal on the other, without having a morphological equivalent in the whole animal kingdom, is certainly too much.

There are thus two points to settle :

1. What was the nature of the pre-existing organ referred to above?
2. Was it paired or unpaired?

It may be remembered that the intra-buccal orifice of the club-shaped gland, which was described and figured for the first time in the paper by Professor Lankester and myself quoted several times above (8), is not present in the very young larvæ, but forms later. This is probably another instance of a slight and unimportant retardation in development.

Before becoming acquainted with the views of van Beneden and Julin I had come to the conclusion, on grounds which seemed sufficient, that the club-shaped gland was a modified gill-slit; and, as I am aware of no other attempts to account for the morphology of the gland, I will give reasons for so regarding it.

In the second place, there are reasons for supposing that it was originally one of a pair of gill-slits, and further, that the original pair actually exists in the larva of *Amphioxus*—the members of the pair being the club-shaped gland and the first primary gill-slit.

If, then, the club-shaped gland is shown to be a modified gill-slit on the one hand, and is homologous with the intestine of the Ascidians on the other, one is led to the apparently preposterous conclusion that the intestine of Ascidians is the morphological equivalent of a gill-slit. The secondary intestine of Ascidians is certainly not homologous with the intestine of *Amphioxus*. No other homology was thought of before van Beneden and Julin suggested that of the club-shaped gland of *Amphioxus*. My observations do not run counter to that suggestion, except possibly in so far as they show that the club-shaped gland is a modified gill-slit. The question is, can it be both?

Reasons for regarding the Club-shaped Gland as a Modified Gill-slit belonging to the Right Side—the Corresponding Slit of the Left Side being represented by the First Primary Slit.

1. The club-shaped gland communicates with the exterior at one end and with the alimentary canal at the other end.

2. Its internal aperture, being dorsal to the larval endostyle, is therefore to the right of the morphological middle line. What was originally a simple branchial passage has thus been virtually drawn out into a long tube, and the external aperture has been shifted from the right to the left side.

3. The first primary slit, being below the endostyle, is therefore to the left of the morphological middle line, and the appearance which it presents of being distinctly behind the gland is superficial and secondary. It lies in the region of the second myotome.

4. The club-shaped gland arises simultaneously with, and in a similar manner to, the first primary slit, as a ventral pouch of the alimentary canal (see Hatschek, 3); and though it arises clearly in front of the first slit, this may be due partly to the remarkable tubular form that it has attained as a result of its evolution. I am not attempting to explain why it passes across to open on the left side, but as it does so it must pass

either in front of or behind the unmodified slit with which it was primitively paired. As a matter of fact, of course, it passes in front of that slit, and so it has come—possibly in some measure owing to the curious torsion which has affected this region—to arise in front of the first gill-slit, with which it really on this view corresponds as a pair. It thus belongs morphologically to the region of the second myotome.

5. The atrophy of the club-shaped gland occurs simultaneously—or nearly so—with the atrophy of the first primary slit. This fact is worthy of the utmost emphasis.

6. In the formation of the secondary slits the first of them pairs with the second primary slit, and no true secondary slit ever appears to correspond with the first primary slit.

7. After the simultaneous formation of the club-shaped gland and the first primary slit (the mouth and anus being also present) there is a prolonged interval, during which no further formation of gill-slits occurs.

This might indicate a distinction between the first primary slit and the succeeding ones.

These are the reasons, drawn from facts, all of which, except Nos. 4 and 7, are illustrated by figures accompanying the present paper.

Deduction.—The club-shaped gland and first primary gill-slit represent an ancestral pair of gill-slits, which atrophies in the course of larval development.

How or for what special object a gill-slit could be modified into such a tubular collateral organ is quite another matter, which I do not now propose to consider further (*vide* p. 210). Suffice it if the reasons given above are enough to lead us to suppose that such a thing has indeed happened in the present case.

The closure of the hinder primary slits presents an interest different from that of the first, and is the decisive step in what I have before called the “symmetrization” of the larva. The transverse growth which carries the more anterior slits from the right to the left side does not extend back so far as the hinder slits, so that they are left in the middle ventral line,



where the endostyle will eventually come to lie; hence the necessity for their closure.<sup>1</sup>

#### MORPHOLOGICAL IMPORTANCE OF THE DEVELOPMENT OF THE ENDOSTYLE OF AMPHIOXUS.

1. Its anterior position in the larva in the part of the alimentary canal which is equivalent to the median portion of the "præchordal" vesicle, shows its entire homology with the endostyle of Ascidians, which is also situated in the median portion of the præchordal vesicle.

2. Its anterior position also supports the view that the endostyle was primitively differentiated in and confined to the mid-ventral line of the ancestral præchordal vesicle.

3. Its oblique position on the right side is secondary, and is due to the rotatory growth which affected the anterior region of the primitive alimentary tract, in correlation with the adaptive forward extension of the notochord.

4. It enables the morphological median line of the larva to be fixed with absolute precision, and shows that it, viz. the median line, has come to occupy a secondary and temporary position, high up on the actual right side of the larva.

5. The important fact can thus be established that the intra-buccal orifice of the club-shaped gland is situated to the right of the morphological middle line.

6. The endostyle of the larva of *Amphioxus* possesses two distinct halves, fused together posteriorly, but with free anterior extremities. At first the right or upper half is much smaller than the lower portion, and this is only natural when one remembers that most of the other structures belonging to the primitive right side are wholly retarded in their development.

7. In the larva it is confined to the region of the first myotome which is destitute of gill-slits.

8. The backward growth of the endostyle of *Amphioxus* has been acquired concurrently with the multiplication of gill-slits in the segmented region of the trunk.

<sup>1</sup> It must be confessed, however, that the meaning of the first appearance and subsequent atrophy of these slits is not quite clear.

9. Since, as will be shown below, the pharynx of *Amphioxus* is not homologous, in the true sense of the word, with the branchial sac of *Ascidians*, the adult position of the endostyle of *Amphioxus* cannot be homologous with that of the *Ascidian* endostyle. The observations recorded in this paper, however, prove unquestionably that the position of the endostyle in the adult *Amphioxus* is secondary, and that in its origin it is, as stated above, perfectly homologous with the endostyle of the *Ascidians*.

### Further Homologies.

It will have been inferred, from what has been stated above, that the pharynx of *Amphioxus*, which is part of the trunk, is not truly homologous with the branchial sac of *Ascidians*, which is part of the præchordal vesicle; so that we have to face the paradox that while a highly specialised organ of (speaking generally) identical structure and relations, and extending the whole length of the pharynx, is present in both cases, yet the pharynx taken as a whole of the one is not homologous with that of the other. This, no doubt, sounds at first like a *reductio ad absurdum* of a morphological problem, but the figures in the plates accompanying this paper, showing the primary and secondary positions assumed by the endostyle of *Amphioxus*, should assist in the realisation of this at first startling paradox.

The gill-slits or branchial stigmata of the *Ascidians* are homologous with those of *Amphioxus* only in the sense that they are distinct vertebrate structures, but they are not homologous in their origin, position, or relations. There is thus a sort of group homology existing between them.

In *Amphioxus* the slits form metamerically in the segmented region of the trunk. In *Ascidians* they form in front of the (however imperfectly) segmented region, and, moreover, van Beneden and Julin point out that they do not arise metamerically one after the other, but irregularly; for instance, the first-formed slit will be the fourth of the series eventually, and so on.

If these conclusions are as sound as they appear to be, it is

interesting to note that there are two kinds of possible homologies, which may be called—

1. Individual homology, the one more commonly met with. This is a morphological factor.

2. Group homology, a physiological factor, but entirely distinct from homoplasy.

It also follows that the peribranchial cavity of Ascidians is in no sense homologous with the atrium of *Amphioxus*; in fact, this is a pure instance of homoplasy.

Bateson (9) points out the probable homology of the proboscis cavity and proboscis pore of *Balanoglossus* with the anterior "præchordal" vesicle and præoral pit respectively of *Amphioxus*; and also he urges the homology between the gill-slits in both cases. These identifications are likely enough to be accurate, but I need not recapitulate the reasons for regarding *Amphioxus* as more closely related to the Ascidians than to *Balanoglossus*, though, since it forms a considerable feature in the present paper, the absence of a true endostyle in *Balanoglossus* might be pointed out. The forward position of the rudiment of a notochord in *Balanoglossus* has probably no relation whatever with the forward extension of the notochord in *Amphioxus*.

As to the affinities of *Amphioxus* with *Ammocætes*, I will only refer to the homology existing between the endostyle of *Amphioxus* and the thyroid gland of *Ammocætes*, which was advocated first by W. Müller and then by Dohrn (7), who worked out the development of the latter structure. It is well known that Dohrn regards the thyroid gland, and therefore the endostyle, as representing a pair of gill-slits. This is of course a side issue, and cannot be fully gone into here; but it may be pointed out that while the thyroid gland gives no reliable indication of a double origin, the endostyle of *Amphioxus* is composed of two distinct halves, which are at first upper and lower respectively, but eventually become right and left—morphologically of course their relation to one another is always right and left.

As for the gill-slits in the two cases, attention may be drawn

to the pair of rudimentary gill-slits, called branchial diverticula, in front of the first pair of true slits, of which Dohrn established the existence in the young *Ammocœtes*, and which never actually open to the exterior; and this circumstance should be remembered in connection with the atrophy of the first pair of gill-slits (i. e. the club-shaped gland and first primary slit) which occurs at a certain stage in the larval development of *Amphioxus*.

Further, the fact that the larva of *Amphioxus* passes through a prolonged critical period, during which it possesses only eight or at most nine pairs of gill-slits, is at least suggestive.

#### ADDENDUM.

The common ancestor of the Ascidians and *Amphioxus* cannot be properly imagined until further knowledge is forthcoming as to the significance of the primary pair of diverticula of the præchordal vesicle and the function of the club-shaped gland.

Gill-slits were probably present in the segmented region of the trunk, which have been lost by the existing Ascidians, but whether one pair or several pairs is a question.

If the club-shaped gland is admitted to be a modified gill-slit, as I hope to have shown that it is, there must have been at least one pair of such slits present.

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## DESCRIPTION OF PLATES XIII—XV,

Illustrating Mr. Arthur Willey's paper on "The Later Larval Development of Amphioxus."

All the figures, without exception, were drawn from the living animal, and each figure is from a separate larva. The drawings are made to scale. From Stage III onwards all the slits are seen through the transparent atrial or body-wall—i. e. they open into the atrium, and not directly to the exterior.

N.B.—The long cilia on the surface of the body are not represented in the figures, nor in those of the memoir by Professor Lankester and myself (8). Their character and position are well shown by Hatschek in his memoir in Claus's 'Arbeiten,' 1881.

FIG. 1.—Stage I. Right side. Fourteen primary slits—notice small size of the first. Six secondary thickenings occurring in a continuous ridge above the primary slits, the first of them being above and between the third and fourth primary slits. The præoral pit of course opens on the left side, and is only seen by transparency on this side.

FIG. 2.—Stage I. Right side. Twelve primary slits. Five secondary thickenings, the last being much smaller than the others, and the first being situated between the fourth and fifth primary slits. The second as well as the first secondary thickening has in this case been slightly retarded; thus showing that the retardation of the first secondary slit, which has been stated in the text to be usual, is not specially important. Of course, the general retardation of development which affects the whole series of secondary slits is extremely important. Notice the endostyle and club-shaped gland.

FIG. 3.—Stage II. Right side. Fourteen primary slits, the fourteenth appearing in side view merely as a pit in the floor of the pharynx; this shows that it is on the way to close up. Seven secondary slits, one having been added behind; the first still lies between the third and fourth primary slits; a new one is subsequently formed in front, so that what appears to be the first slit or thickening at this stage eventually becomes the second secondary slit. In this larva the second to the sixth of the secondary thickenings have just been perforated. The appearance of a seventh thickening behind is rather early to happen at this stage.

FIG. 4.—Stage III. Right side. Twelve primary slits; the first of them is getting smaller. The fin-chambers seem to form about this stage. There is a small anterior portion of the atrium still unclosed. The right wall of the future velum is now seen from this side, owing to the great extent to which

the mouth has bent round the anterior extremity of the alimentary canal. There are seven secondary slits, the seventh not yet perforated; the first is still above and between the third and fourth primary slits. Notice the flattened tops of the larger secondary slits. All the primary slits behind and including the fourth now bend under the pharynx towards the left side. Notice the endostyle and club-shaped gland.

FIG. 5.—Stage IV. Right side. Thirteen primary slits, the thirteenth closing. Seven secondary slits, the first and last having only just opened. The true first secondary slit has now appeared, and lies above and between the second and third primary slits. The larger secondary slits have begun to double in to form the tongue-bars. Notice the direction of the long axis of the secondary slits. All the primary slits are now bending under the pharynx, and the first is extremely small. The endostyle has definitely commenced to grow beyond the club-shaped gland. This should be noticed carefully and compared with the condition of the two structures in the preceding stages. The atrium is quite closed in front.

FIG. 6.—Stage IV. Left side. This is to show the extent to which the primary slits have come round to the left side at this stage. The number—fourteen—of primary slits is not typical for this stage (see Fig. 5). The right or anterior half of the velum is seen with a deep focus. There are six elements of the buccal cirri in the lower half of the oral hood, growing out into tentacles and covering up the external orifice of the club-shaped gland. The upper half of the oral hood is hanging over the upper wall of the mouth or velum and over the præoral pit.

FIG. 7.—Stage V. Right side. This is the very commencement of Stage V; and the first primary slit, though very small, is still to be seen in side view. The secondary slits, of which there are eight, have become larger; and in the third slit the tongue-bar has actually fused with the ventral wall of the slit. The endostyle and club-shaped gland have the same relations as in Fig. 5. The cæcum is commencing to grow out; there is no fixed stage at which this occurs. The primary slits have nearly disappeared from this side, but are still seen below the secondary slits.

FIG. 8.—Stage V. Left side. The primary slits are not fully round to the left side yet, but the larger of them are commencing to form tongue-bars. The twelfth primary slit is very small, and the thirteenth and fourteenth are closing. Notice the point of junction of the upper and lower portions of the oral hood; the latter is prolonged anteriorly as a ridge. The right wall of the velum is deeply focussed.

FIG. 9.—Stage V. Three-quarter ventral view. This shows the first primary slit in a condition of approaching atrophy. The twelfth is also closing. The bases of the secondary slits can just be seen.

FIG. 10.—Stage V. Ventral view. This should attract particular attention.

Feeble traces of the first primary slit can just be detected in front. The twelfth primary slit is again seen to be closing. Notice the series of primary slits curving round to the mid-ventral line behind. The tongue-bars are complete in most of the secondary slits, but in none of the primary slits.

FIG. 11.—Stage VI. Left side. Nine (primary) slits, the first being of course originally the second primary; but it has come to be the first in position in consequence of the atrophy of the true first. Behind there are traces of the eleventh and twelfth primary slits. Only the left half of the velum can now be seen from this side. I do not know what has become of the nephridium of Hatschek;  $x$  is probably part of the ciliated tract of the præoral pit. Notice the left half of the endostyle which extends under three slits; the right half is out of focus. In this larva there were eight secondary slits on the right side.

FIG. 12.—Stage VI. Left side. This shows the twelfth and thirteenth primary slits in course of closure. The condition of the gill-slits is not quite so far advanced as in the preceding figure, but the buccal cirri are farther advanced. There were seven (secondary) slits on the right side.

FIG. 13.—Stage VII. Left side. In this larva the ninth slit (= tenth primary) would probably have closed. The eleventh primary slit is represented by a rudiment. Cæcum is commencing to bulge in the region of the twentieth myotome. Notice the length of the endostyle. The buccal skeleton is beginning to grow into the upper portion of the oral hood.

FIG. 13*a*.—Separate view of the velum of the same larva, showing the four primary velar tentacles. The right and left halves of the velum are seen to be not quite opposite to one another.

FIG. 14.—Stage VII. Right side. The slits are commencing to elongate in a direction at right angles to long axis of body. There are eight (secondary) slits on this side. On the left side of this larva there were eight (primary) slits, and there was also a median rudimentary slit on the point of closure, viz. the tenth primary, which sometimes persists, as explained above, as the ninth slit of the left side. The view of the endostyle is very important, showing its double nature—upper and lower halves. It has grown considerably in length. The right metapleur is omitted. The floor of the atrium consists of the expanded subatrial ridges.

FIG. 15.—Stage VIII. Right side. In this larva there were eight slits on each side and no rudimentary one behind, so that this is typical of the critical period. The view of the endostyle is again very important. The two halves have now assumed a definite right and left position. Its double origin can be detected here for the last time. Notice in this and the preceding figures the hyperpharyngeal band. Right metapleur is omitted.

FIG. 16.—Stage VIII. Left side. Nine gill-slits; the ninth has not fully come round to this side, but bends under the pharynx. There were eight slits

on the right side. Notice the cœlom just in front of the cœcum. The blood-vessel in this region is contractile. The endostyle is farther advanced than in the preceding figure. The right and left walls of the velum are quite opposite to one another.

FIG. 17.—Stage VIII. Combination of three focussings, showing the last (seventh) pair of slits of a larva in which the ninth primary slit was in a rudimentary condition in the mid-ventral line.

FIG. 18.—Stage V. Right side (anterior portion only), to show the endostyle, which has grown a long way past the club-shaped gland between the primary and secondary slits.

FIG. 19.—Stage VI. Similar view of right side, to show that the club-shaped gland has now atrophied, its remains being still visible on the endostyle.

FIG. 20.—Stage I. Left or oral aspect of a larva. Note pointed anterior extremity of mouth, ciliated groove, external orifice of club-shaped gland, and one element of buccal skeleton.

FIG. 21.—Stage I. Similar view. Antero-dorsal margin of mouth, commencing to hunch up into the ciliated groove. Two elements of buccal skeleton.

FIG. 22.—Stage II. Similar view. Hunching up and sinking inwards of the anterior portion of the mouth continued. Three elements of buccal skeleton.

FIG. 23.—Stage II. Similar view. Four elements of buccal skeleton, commencing to grow out as tentacles. When the very young cirri are seen end-on they present a circular appearance.

FIG. 24.—Stage II. Similar view. Five elements of buccal skeleton.

FIG. 25.—Stage III. Similar view, to show the anterior half of the mouth bent at a deep focus towards the right side. Note also the two portions—upper and lower—of the oral hood, the latter containing the buccal skeleton.

FIG. 26.—Stage IV. Similar view. This is an important view of the mouth, showing its change in shape but not diminution in size. The external orifice of the club-shaped gland is covered over by the buccal cirri.



# On the Structure of Two New Genera of Earthworms belonging to the Eudrilidæ, and some Remarks on Nemertodrilus.

By

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With Plates XVI—XX.

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THE worms which form the subject of the following paper were obtained in a living state from Kew Gardens, along with a number of others whose structure I propose to investigate later.

I had applied to Mr. Thiselton Dyer for leave to sift soil coming from tropical countries in the Wardian cases which are generally used for transmitting plants in pots. This Mr. Dyer very kindly permitted me to do, and in his absence Mr. Morris was so good as to put me in the way of carrying out my wishes. To both these gentlemen my thanks are tendered. In the earth surrounding a number of pots containing plants from Lagos, West Africa, I found about half a dozen worms, which proved on examination to belong to the family Eudrilidæ.

Until quite recently one genus—Eudrilus itself—was the only known representative of the family; three years ago Dr. Rosa described from Scioa, Africa, another genus—Teleudrilus; and quite recently Dr. Michaelsen has received from Zanzibar and the mainland opposite, and from the mouth of the Zambesi River, a number of species, all belonging to this family, and referable to four new genera: the family is, therefore, in the present state of our knowledge, characteristically Ethiopian,

though the type genus *Eudrilus* has not yet been met with from the African continent.<sup>1</sup>

The *Eudrilidæ* which I obtained from Kew belong apparently to two distinct species, which are also generically distinct. Four specimens I refer to a new genus, for which I propose the name of *Hyperiodrilus*. The fifth specimen is a species of another genus—*Heliodrilus*, nov. gen. The sixth is a very minute worm, measuring barely an inch in length, and being sexually immature was indeterminable.

### I.—*Hyperiodrilus africanus*, nov. gen., sp. nov.

The worms are of different sizes; the largest specimen measures (after preservation in spirit) about five inches; during life the length was rather greater. Their form is very slender, and their movements were active, though they did not show any power of jumping, such as is shown by *Perichæta*. While moving the buccal cavity is to a certain extent everted, and is made use of as a sucker for attaching the front end of the body; the eversion of the buccal cavity is not nearly so pronounced as in *Perichæta*.

The colour of this species is pinkish, and the posterior segments have a distinctly ringed appearance. There does not seem, however, to be much pigment in the skin; the colour is entirely due to the enclosed viscera, particularly, of course, to the blood-vessels—even the ringed appearance of the posterior segments is due to the same cause; the blood-vessels ramifying over the septa produce the appearance of bands of red pigment corresponding to the segments: the clitellum is yellowish.

#### § External Characters.

An examination of the external characters at once shows that this worm is referable to the *Eudrilidæ*, but that it cannot be included in any known genus of that family, with the possible exception of *Stuhlmannia*.

<sup>1</sup> Since the above was written Drs. Horst and Michaelsen have received from Africa specimens of *Eudrilus*.

The prostomium does not completely divide the peristomial segment; but it sends back (see fig. 6) a very narrow prolongation, which is embedded in the peristomial segment up to a point not very far distant from its posterior border. Such a narrow prolongation of the prostomium appears to be characteristic of the Eudrilidæ; it is specially mentioned by Rosa (10) in the case of *Teleudrilus*, and is figured by Michaelsen (7, pl. iii, fig. 17) in *Nemertodrilus*.

The dorsal setæ are in couples; of the ventral setæ, the individual setæ of each couple are some little way apart, as illustrated in figs. 9 and 12.

The clitellum is developed all round the body, and occupies four segments, Nos. 14, 15, 16, and 17, as in other Eudrilidæ, where four segments is also the usual extent of the clitellum, though sometimes, as in *Polytoreutus*, exceeded. Only one specimen, however, had the clitellum developed upon the 14th segment, and here it was incomplete, extending only over the dorsal surface of that segment. In two other specimens the clitellum only occupied Segments 15, 16, 17.

Nephridiopores were obvious upon most of the segments of the body, particularly upon the clitellum, where the smooth swollen integument rendered them very easily visible. They lie almost in the intersegmental furrow in front of the dorsal setæ. It may be noted that they occur, as shown in fig. 23 of Pl. XVIII, in the 14th and 17th segments, where the ducts of the generative organs open.

Dorsal pores could not be detected in any part of the body.

The apertures of the oviducts are upon the 14th segment near to its posterior border: each has the appearance of a minute hemispherical projection, which cannot be confounded with a nephridiopore; besides, as already mentioned, the 14th as well as the 15th segment has its nephridiopores.

The oviducal papillæ, as they may be more accurately spoken of, are situated behind the dorsal seta of the 14th segment on each side; in one case, as shown in fig. 23 of Pl. XVIII, they

were placed just in front of the nephridiopore of the segment behind; more usually, however, they are a little dorsal of the nephridiopore.

The aperture of the sperm-ducts is, as in *Stuhlmannia* and *Teleudrilus*, single and median; it lies at the end of the clitellum, between the 17th and 18th segments. It does not, however, present the appearance of an orifice, but of a prominent hemispherical papilla, as in the case of the oviducal pore; the papilla is in this case considerably larger.

In one specimen (that which had four clitellar segments) this papilla was connected by two grooves running obliquely forwards with a pair of conspicuous rounded papillæ, placed near to the boundary line between the 17th and 16th segments; the two grooves diverged from each other at an angle of about  $40^{\circ}$ ; each ran along a slight mound-like elevation, as shown in fig. 24 of Pl. XVIII, and more highly magnified in fig. 19 of the same plate. The two papillæ are unsymmetrically disposed, as also shown in the figure; the left-hand one is placed just behind the groove separating the 17th from the 16th segment; the right-hand one at about the middle of the 17th segment.

In a second specimen (Pl. XVIII, fig. 22) the median papilla was quite invisible, owing probably to the worm having died with the part in question retracted.

In the only other specimen belonging to this species, which was nearly mature, a prominent glandular swelling occupied the middle ventral line between Segments 17 and 18 (Pl. XVIII, fig. 20). A careful examination showed that the apparently single protuberance is not to be confounded with the median papilla described in the first of the three specimens; it really represents the two anterior papillæ of the 17th segment closely fused; the groove issuing from each may be detected, and the median papilla itself is visible at the point where the two grooves nearly come into contact.

In *Stuhlmannia variabilis* (Michaelsen) the papillæ connected with the male efferent apparatus show certain resemblances to those of the present species.



There is a median aperture (as in *Teleudrilus* and other *Eudrilidæ*) of the "prostate glands" upon the 17th segment; this is spoken of as a slit-like orifice; but the apparent difference in this particular from *Hyperiodrilus africanus* may be a mere question of the state of contraction of the worm's body. From the aperture upon the 17th segment a deep furrow runs forward to a process which bears the outlet of a peculiar gland; this process is median, but slightly inclined to the right side. This papilla, however, appears from Michaelson's description to lie upon the 13th segment, near to the ventral unpaired orifice of the spermatheca. As already mentioned, the lateral processes, though varying somewhat in their position upon the segment, are always on Segment 17.

On the 13th segment is a median aperture (see fig. 24) corresponding to the male generative orifice upon the 18th segment, though situated at about the middle of the segment, rather nearer to the anterior than to the posterior boundary. This aperture was not at all conspicuous upon any of the specimens examined, and might very easily be overlooked.

### § Integument.

The layers of the body-wall are as in other earthworms, except for the presence in the epidermis of certain peculiar organs, which appear to be met with in all *Eudrilidæ* except *Nemertodrilus*; but this genus is in other respects (see p. 266) a very aberrant member of the family. These structures are described later.

The muscular layers of the body-wall show no noteworthy peculiarities as regards the structure and arrangement of the fibres. The longitudinal coat does not exhibit the bipinnate character which is frequently met with in earthworms, particularly in the *Lumbricidæ*.

The muscular coats contain numerous irregular spaces filled with cœlomic corpuscles (see fig. 3); besides the ordinary corpuscles large multinucleate bodies are met with, which may be pathological formations. I believe that Kühenthal (13) was the first to specially call attention to the spaces

in question. They are unusually abundant in both *Hyperiodrilus* and *Heliodrilus*—more so than I have observed in any other earthworm. In places each individual fibre was separated from its neighbours by quite large spaces filled with corpuscles.

### § Setæ.

The arrangement of the setæ is somewhat peculiar; the more dorsally situated pair, which are in reality lateral, and not dorsal, are closely approximated; the ventral couple (fig. 12) are at some distance apart; the distance separating the 2nd from the 3rd seta<sup>1</sup> is twice that which separates the 1st from the 2nd. The arrangement of the setæ is such as to produce the impression that there are only three on each side of the body. It appears to me possible that Kinberg's genus *Tritogenia* has the setæ arranged in this way, having thus led Kinberg to make the statement that it possessed only six setæ per somite. Perrier, who has (9) re-examined this genus, has discovered that there are eight setæ in each segment; the position of the male generative pores of *Tritogenia* between the 16th and 17th segments (according to Kinberg) is another point of similarity between the two genera, which are very likely identical.

No other known genus of Eudrilidæ has the setæ arranged in this way; there appears, however, to be a slight difference in *Nemertodrilus griseus* in the distance which separates the individual seta of each couple; this is a step in the direction of *Hyperiodrilus*.

### § Epidermal Sensory (?) Organs.

The epidermis of this worm is furnished with certain curious structures of doubtful nature, identical with those which I was the first to describe in *Eudrilus*.

As Rosa has indicated the presence of these structures in *Teleudrilus*, and as I have found them in *Heliodrilus*, they may be regarded as characteristic of the Eudrilidæ, and, as far as our present knowledge goes, confined to that

<sup>1</sup> The first seta is that nearest to the nerve-cord.

family as defined by myself.<sup>1</sup> This, although a small point, is an additional argument in favour of retaining that family within the limits which I have proposed for it.

The structures in question are visible in the epidermis when the body-wall is examined as a flat preparation in glycerine; and they may be observed to be scattered irregularly over the segments, thus affording an example of another system of organs which have no perceptible relation to the metamerism of the body. In a preparation of this kind the sensory organs appear as longitudinal furrows, longer than broad, arranged after no system that I could discover, save that they were absent upon the intersegmental furrows. In transverse sections of the body-wall (cf. fig. 3) the sensory organs are seen to lie in the epidermis, but not to reach its surface; they generally cause the membrane which separates the epidermis from the underlying layer of transverse muscles to be bulged out towards the latter. Above each body is a row of short epidermic cells which divide it from the cuticle. The real form of the sensory bodies is better seen in longitudinal sections, for they lie for the most part parallel with the long axis of the body of the worm.

In such a section each sensory body is seen (see fig. 2) to consist of a central cylindrical core faintly stained by borax carmine, in which are embedded a variable number of large oval nuclei. Round the axis are a series of coats like those of an onion, which seem to be composed of an elastic membrane; in such sections, and in the transverse sections also, these coats appear as highly refracting fibres. In fragments of the skin mounted entire and viewed from above, the membranes present the appearance of a series of fine striæ surrounding the axis of the body. Between the several membranous coats are darkly staining nuclei which are quite distinguishable from those which lie in the axis by their very much smaller size; the central nuclei are fully twice the size of the peripheral nuclei.

<sup>1</sup> In a forthcoming résumé of the classification and distribution of Earthworms to be published in the 'Proceedings of the Royal Physical Society.'

These bodies have a striking resemblance to certain "end organs" which are found among the Vertebrata; they are particularly like the Pacinian bodies, having the same concentric lamellæ surrounding a central sheath. It is this resemblance which makes me believe that the structures in question are of a nervous nature, for I must confess to having found no unmistakable evidence of nerve-fibres connected with them. It is, however, not easy to trace the ramifications of nerves in the skin of earthworms which have been preserved with alcohol.<sup>1</sup>

### § Alimentary Canal.

In its main features the alimentary canal agrees with that of *Heliodrilus*, to be presently described.

The pharynx is large, and extends back to the 6th or 7th segment; there is no anteriorly situated gizzard, but a series of five or six, each occupying a single segment at the junction of œsophagus and intestine.

The œsophagus is lined with a thin chitinous cuticle as far back as the opening of the calciferous glands; beyond this point its walls are ciliated.

### § Calciferous Glands.

As in *Eudrilus*, and apparently other *Eudrilidæ*, the present genus is furnished with two kinds of calciferous glands.

(1) A pair of voluminous glands are attached to the œsophagus in Segment 13, the cavity of which they largely fill. The œsophagus itself is very narrow in this region, scarcely wider than the dorsal blood-vessel, which like it is completely hidden by the large glands. These glands appear of a reddish-purple colour in the spirit-preserved specimen, the colour being of course due to the abundant blood-spaces interspersed among the tissue of the glands. The glands have a trifid appearance as in *Eudrilus*, and a large blood-vessel passes over each.

In transverse sections these paired calciferous glands are

<sup>1</sup> Since the above was written Dr. Horst in a paper cited on p. 252, footnote, has suggested the sensory nature of the problematical structures.



seen to consist of numerous folds of epithelium with blood lacunæ lying between the epithelial layers; the secretion of the glands has the form of small spherical particles.

(2) The present genus, like other Eudrilidæ, possesses impaired ventral diverticula of the œsophagus.

The occurrence of these structures was first put on record by myself in *Eudrilus sylvicola* (1), where they are simple diverticula with the lining epithelium thrown into a few longitudinally running folds. Rosa (10) mentions the presence of three such glands in Segments 9, 10, 11, in *Teleudrilus*, but gives no special description of them.

To Michaelsen (7) our chief knowledge of these peculiar glands is due. He terms them "Chylustaschen," and compares them with certain glandular diverticula in the Enchytræidæ. The function of these structures is considered by Michaelsen to be not analogous to that of the calciferous glands; instead of secreting calcareous granules like the calciferous glands, or producing any other kind of secretion, it is supposed that they serve for the absorption of food—hence the term "Chylustaschen." It must be admitted that no calcareous spherules have been found in these pouches, although their structure recalls that of the calciferous glands; so far this is evidence, if not in favour of the suggestion of Michaelsen, at any rate of their performing a different function from that of the calciferous glands in the economy of the worms.

In *Pygmæodrilus* the 9th segment contains a pair of lateral forwardly directed diverticula; it may be that the impaired glands of *Eudrilus* are formed by the fusion of a pair which get to be more and more approximated ventrally: on the other hand, the diverticula of *Pygmæodrilus* rather suggest the calciferous glands of *Urochæta*, which really secrete calciferous particles.

No such structures are mentioned in *Eudriloides* or *Nemertodrilus*.

In *Polytoreutus* there are median impaired pouches as in *Hyperiodrilus*, three in number, besides the paired calciferous glands; the folds in the interior of the glands are so

complicated as to present the appearance of a bundle of longitudinally running vessels.

In *Hyperiodrilus* the ventral œsophageal pouches are in certain respects more remarkable. There are three of them in Segments 9, 10, and 11—one pouch to each segment.

The pouch appears to communicate with the œsophagus a little to the right of the ventral median line. The orifice is narrow, and the cells are at first identical with those which constitute the epithelial lining of the œsophagus; the cells are tall and columnar, and between their bases are spherical or pear-shaped darkly staining cells (fig. 31) like those of the œsophagus. Tracing the pouch back, the cells are seen to alter their character, and to become low and quadrangular in form, while the epithelium is so folded as to give the appearance of a series of tubes running approximately parallel to each other (fig. 25). At the point of origin of the pouch, as shown in fig. 31, the muscular wall of the œsophagus and the peritoneal covering becomes reflected over the gland in such a way as to leave a wide space between itself and the pouch; further back this space is obliterated by the coalescence of the muscular and peritoneal layers with the outer layer of the gland.

Further from the point of the opening of the gland into the intestine the appearance of parallel running tubes is increased, and they have become at the same time of smaller calibre. The cells which form the lining epithelium are broad and somewhat flat, though quadrangular in form; the nuclei are thus, owing to the large size of the individual cells, very far apart, and the cell outlines are not distinguishable. As the folding gets more and more complicated the "tubes" present more and more the appearance of having an intra-cellular lumen; this actually does take place at the extremity of the pouch. One of the last sections through a pouch is illustrated in fig. 26: it will be seen there that three comparatively wide tubules end in a complicated meshwork of fine capillary tubes; the section presents the strongest possible resemblance to a portion of a nephridial network such as I have figured in *Acanthodrilus multiporus*. Even the three large tubes

figured in that drawing appear to be excavated in the substance of cells, while there can be no possible question about the ramification of minute tubes.

One is tempted to regard every ductule excavated in the substance of cells as of nephridial nature, but the origin of the blood-capillaries in the leech by direct canalisation of cells, discovered by Lankester and confirmed by Lang, as well as unicellular glands with a central duct, shows that this conclusion cannot be always drawn.

The study of these œsophageal pouches in *Hyperiodrilus* is instructive as showing how folds may gradually acquire the character of a system of tubes, and how the subdivision of the tubes, without a corresponding decrease in size of the epithelium, may ultimately lead to an intra-cellular network. This series of facts shows also how irrational is the distinction, which some have attempted to draw, between nephridia with an intercellular duct and nephridia with an intra-cellular duct, e. g. between those of the *Polychæta* and *Oligochæta*.

The tubes are everywhere separated by an abundant plexus of blood-capillaries, which seems to form a continuous sinus.

In *Bucholzia* there is a dorsal diverticulum of the œsophagus, which Michaelsen has compared with the ventral pouches of *Eudrilus*; and in this *Enchytræid* the diverticulum is formed of a number of tubules with intra-cellular lumina.

### § Generative Organs.

1. Male Generative Organs.—I could only find a single pair of testes; as these were extremely small, it is possible that I may have overlooked the second pair. The pair found were in the 11th segment; each is attached to the vas deferens just where it issues from the septum separating this segment from the one in front. As will be seen later, after the other organs of the male reproductive system have been described, the peculiar arrangement of the vasa deferentia, which are bent upon themselves near to their cœlomic opening, suggests that the missing pair of testes, if they are really present, will be found in the 12th segment.

The testes of *Hyperiodrilus* are not enclosed in a special

sac, as they are in *Heliodrilus*. The seminal cells must therefore trust to accident to find their way into the interior of the sperm-sacs.

There are two pairs of sperm-sacs (fig. 44) in Segments 11 and 12. Each depends from the anterior of the two septa which bound the segment by which it is contained. The sperm-sacs are not very large, and are perfectly independent of each other. The sperm-sacs are shaped something like a bean, the hilum being the point of attachment to the septum by means of a short pedicle. The interior of each sperm-sac is divided up by numerous trabeculæ into a series of very small cavities, which contain decidedly more gregarines than developing spermatozoa.

The vasa deferentia present close resemblances to those of *Teleudrilus* (Rosa, 10). The funnel opens into the sperm-sac, and therefore traverses the septum twice, since the sperm-sacs lie on the posterior aspect of the septa separating Segments 11, 12, and 10, 11 respectively. This is precisely what occurs in *Teleudrilus*, and I have recently pointed out that in a species of *Moniligaster* there must be something of the same kind, inasmuch as the funnel projects into the sperm-sac which is attached to the front wall of its segment. Generally when the sperm-sacs are attached in this way the funnel of the vas deferens is not in direct continuity with them, but projects freely into the interior of the segment a little way in front of the posterior septum of the segment.

The vasa deferentia open in the way that has been described by four rather small funnels completely concealed within the four sperm-sacs. On leaving the sperm-sac the vas deferens is at first a somewhat narrow tube, lined by numerous small quadrangular cells, which are of course ciliated; the peritoneal covering is slight, and there is no such conspicuous muscular coat as I have figured and described in *Eudrilus*. Almost immediately the vas deferens widens out exactly as in *Teleudrilus*, and is sharply bent upon itself, and again traverses the septum; directly it has passed through the septum it narrows. The wide U-shaped portion of the vas



deferens is lined by a tall columnar epithelium, and the cilia are very long. The narrow portion lying behind the septum is composed of an epithelial layer of low quadrangular cells comparatively few in number; the two vasa deferentia of each side pass down the body just covered by the peritoneum, and on a level with seta No. 3; they are accompanied by a blood-vessel which supplies them with capillary branches.

In the 17th segment is situated the terminal apparatus of the male reproductive organs, which consist of two large "prostate" glands or atria, opening on to the exterior by means of the protrusible penis.

Each atrium is furnished with a muscular duct which leads to the exterior.

The entire gland is sausage-shaped, and recalls the corresponding structure in *Acanthodrilus*; it has the same form as in that genus, and the same opaque white appearance. We do not find the nacreous appearance of these organs in *Eudrilus*; the reason for this difference is to be found in the absence from *Hyperiodrilus* of the thick muscular coats composed of longitudinal and circular fibres which are characteristic of *Eudrilus*. In *Hyperiodrilus* the muscular layer is indeed present, but it is reduced, as shown in fig. 38, to a very thin layer. This figure may be compared with that illustrating a transverse section through the atrium of *Eudrilus* (Beddard, 1, pl. xxx, figs. 8—10).

The interior of the distal part of the prostate is formed of a compact mass of cells loaded with darkly staining granules. I could not distinguish (see fig. 42) two layers of cells, such as appear to be met with in the prostates of all other earthworms in which those glands have the tubular form which they exhibit in the present genus; that this, however, is due to the obliteration of the distinction into two layers by the immense quantity of secretion present is shown by another specimen (fig. 38), in which the inner layer of columnar cells was quite plainly visible.

The vasa deferentia, which retain their distinctness up to the very point of opening, open into the glandular part of the

prostate nearer to the distal than to the proximal end. The direct communication between the vasa deferentia and the "prostate" appears to be the rule with Eudrilidæ; and this led me to compare the so-called prostate in the terrestrial Oligochaeta to the atrium of the Limicolous forms. Quite recently Benham (6) has expressed himself against this identification, but admits that "a portion of the prostate in Perichæta, Eudrilus, and other genera, in which the sperm-duct and prostate form is probably the homologue of the 'atrium' of Tubifex." In this case half of the glandular portion of the terminal organ of the vasa deferentia in Eudrilus, Hyperiodrilus, &c., will be the equivalent of the "atrium," while the other half will be comparable to the "cementdrüse" of Tubifex! This will hardly do, for there is no structural distinction between the two halves; they form a continuous whole. I need not recapitulate here the various intermediate conditions which unite the Eudrilidæ with other genera; it appears to me impossible to draw a line between the "prostate" glands of Perichæta and those of Eudrilidæ; they are obviously homologous structures. The name that is applied to one must be applied to the other.

There are no penial setæ present.

2. Female Generative Organs.—These organs seem to be most like those of *Stuhlmannia variabilis*, which have been briefly described by Michaelsen as follows:—"The orifice of the spermatheca situated in the middle ventral line of Segment 13 leads into a wide atrium. From this is reached an unpaired long sac-like crinkled spermatheca. From the atrium arises on each side another spermatheca-like broad canal. These two canals extend upwards and fuse together above the gut, forming in this way a single short sac, which communicates with the atrium by a ring-like canal surrounding the gut. Two greatly coiled oviducts, each furnished with a receptaculum ovarum, open laterally on Segment 14. On the other side they communicate with the spermatheca. The two ovaries lie anteriorly in Segment 13. (They are connected by narrow canals with the oviducts?)"

On dissecting a specimen of *Hyperiodrilus*, the only part of the female reproductive system that could be at first detected (fig. 47) was a longish oval spermatheca lying upon the dorsal wall of the gut, in the 13th segment, and directed backwards. After carefully removing the calciferous glands, to which the spermatheca and neighbouring part of the reproductive organs are closely attached, the spermatheca was seen to divide into two thick-walled tubes; these (see fig. 1) are placed close to the septum which divides the 13th from the 12th segment, and which is conspicuous on account of its being the last of the specially thickened septa. The two tubes, as in *Stuhlmannia*, completely encircle the gut, and meet below in the small atrium which opens on to the exterior by the median pore already referred to as existing upon the 13th segment. Each of these two tubes is provided on the outer side (fig. 8) with a small prominence, which looks like a diverticulum of it, and which corresponds to the structure termed by Michaelsen "receptaculum ovarum" in *Stuhlmannia*; close to this arises on either side the oviduct, which passes straight to its opening on the 14th segment.

The atrium is not furnished with a second diverticulum corresponding to the "spermatheca" of *Stuhlmannia*. Fig. 47 represents the parts described as seen on the dissection of the worm. In fig. 8 is a more diagrammatic sketch of the same parts, in order to display their mutual relations, and their position with regard to the œsophagus and the dorsal blood-vessel.

An investigation by means of longitudinal and transverse sections shows that these structures, which are alone clearly visible on a dissection, and which form a continuous whole easily to be separated and mounted on a slide, do not represent the entire female reproductive system.

The ovaries are paired structures, lying as usual in the 13th segment; they are very compact bodies, not frayed out into numerous processes. Instead of lying freely in the cavity of the 13th segment, each ovary is enclosed in a special cœlomic sac of a globular form; this sac also includes a

portion of the nephridium of the 13th segment (fig. 10) ; its walls are made up of muscular fibrils, and it has a coating as well as a lining of peritoneal cells. Each sac is situated near to the anterior wall of the 13th segment ; traced backward by means of a continuous series of transverse sections, the sac abruptly diminishes in calibre, and forms a narrow tube which is continuous with the tube formed by the narrowing of the ovarian sac of the opposite side of the body. A section which illustrates these relations is illustrated in fig. 51.

The bursa copulatrix, which is spherical in transverse section, opens on to the exterior by the median pore of Segment 13. From the bursa copulatrix arises a single blind pouch, which may be regarded as the spermatheca. This has a lining of tall epithelial cells of a glandular appearance, and very thick muscular walls. The spermatheca directly it leaves the bursa becomes enveloped in a cœlomic sac, as shown in fig. 11 ; this cœlomic sac is not the connecting tube between the two ovarian sacs shown in fig. 51, but it is continuous with the sac involving the ovary of its own side ; the spermatheca of each side runs up the side of the œsophagus for a short way, terminating blindly at about the middle of the dorso-ventral diameter. The sac in which the spermatheca is contained passes right round the œsophagus, and, fusing with its fellow of the opposite side of the body, is prolonged backwards as an unpaired median sac lying above the œsophagus ; this structure is that which is illustrated in figs. 5, 11, and lettered *sp'*.

It must be noted, therefore, that what appears on dissection to be an unpaired spermatheca, lying above the œsophagus and connected with the bursa by a ring round the œsophagus, is really a cœlomic sac containing the true spermatheca, and does not communicate directly with the exterior through the bursa copulatrix.

This cœlomic space comes into close relations with the oviducal funnel which seems to open into the receptaculum ovarum (figs. 1, 5, 8, 11 *r. o.*), but does not involve the receptaculum or the oviduct.



The receptaculum, or egg-sac, as it may be more simply termed, is of considerable size, and is divided up into numerous compartments, which lodge the developing ova, by trabeculæ; it is closely attached to the periesophageal cœlomic sac, as shown in the figure (fig. 10), and in all probability opens into it, though I confess to not having been able to find the actual orifice of communication.

The oviduct is a short, straight tube, which passes directly from its opening into the egg-sac to the external aperture upon Segment 14. It is lined by a columnar ciliated epithelium, and it has strongly developed muscular walls.

The cœlomic sac involving the ovaries, and continuous with the ring round the œsophagus, facilitates the passage of ova from the ovary to the receptaculum and to the oviduct; but the extension of the sac beyond the limits necessary for that purpose is a little difficult to understand.

I could not find any spermatozoa in the dorsal sac, nor, for the matter of that, in the spermatheca; and if impregnation takes place by the bursa copulatrix, there seems to me to be no way by which the spermatozoa could reach the interior of the sac. I could detect no orifice leading from the spermatheca to the sac which involves them, and such a connection seems hardly likely to occur.

In nearly all the Eudrilidæ the ovary is contained in a special cœlomic sac, which communicates indirectly with the exterior. The principal exception is *Nemertodrilus*; but here the reduction in size of the cavity of the 13th segment seems to guide the ova to the aperture on to the exterior of the body, and there is thus no need of the formation of a special tube (see p. 266).

The remarkable genitalia of *Polytoreutus* are perhaps rendered more intelligible by the facts which I have been able to make out in *Hyperiodrilus*. I have little doubt that the median sac, communicating on the one hand with the "spermatheca," and on the other with the oviduct, will prove to be a cœlomic space perhaps surrounding the true spermatheca. It seems to be possible that the female organs of

*Teleudrilus* will bear reinvestigating from this point of view. The two tubes which are described by Rosa (10) as diverticula of the spermatheca have a certain likeness to the narrow cœlomic sac of *Hyperiodrilus*, which puts into communication the ovarian sacs and the space surrounding the spermatheca; and they approach each other in the middle line, which suggests the possibility of their being really fused.

The mature ova have the characters illustrated in fig. 49. The most remarkable feature is a thick, darkly staining membrane, which completely surrounds the ovum; this membrane is traversed by numerous pores.

I have described something of the same kind in *Eudrilus* (2), where, however, it forms a cap at one end only of the ovum. After examining *Hyperiodrilus* it seemed possible that I was mistaken in considering that the membrane in question was limited to half of the ovum in *Eudrilus*. I cannot, however, find that I have made any mistake in this matter in my description of *Eudrilus*.<sup>1</sup> This being so, it does not seem very likely that the cap of darkly staining cubical bodies which cover one pole of the ovum in *Eudrilus* are comparable to a radiately striated egg-membrane; such a membrane would surely be produced round the whole of the periphery of the ovum at once. There are, therefore, still reasons for adhering to the opinion which I expressed in the paper dealing with the structure of that ovum, viz. that the columnar layer is in reality a product of the follicular cells, being formed by the metamorphosis of a certain number of them. This opinion is considered by Vejdovsky (12) to be probably true.

The resemblance between this structure in *Eudrilus* and the complete membrane which surrounds the ovum of *Hyperiodrilus* is close; but I do not feel sure that they actually correspond; a membrane surrounding one half of the ovum seems to be exceedingly anomalous. The ova within

<sup>1</sup> My description has been confirmed in a paper by Dr. Horst which I received after the present memoir was sent to Professor Lankester, "Sur quelques Lombriciens exotiques appartenant au genre *Eudrilus*," 'Mém. Soc. Zool. de France,' t. iii, p. 223 (cf. fig. 11 of plate).

the compartment of the egg-sac are associated with a few germinal cells, which are generally closely attached to the ovum, and frequently show signs of degeneration. The masses of immature germinal cells and ova, in all stages of development, which I have figured and described in *Eudrilus* (2) are not to be found in the present genus.

## II.—*Heliodrilus lagosensis*, nov. gen., n. sp.<sup>1</sup>

Among a number of earthworms which arrived in a Wardian case at Kew Gardens from Lagos, West Africa, was a single specimen which I refer to a new genus, belonging, like *Hyperiodrilus*, to the family Eudrilidæ.

### § External Characters.

It is of about the same size and colour as *Hyperiodrilus*, but the external characters of the specimen when killed and preserved are quite unlike those of the former species.

The prostomium is of the same form as that of *Hyperiodrilus*.

The setæ have precisely the same arrangement as in *Hyperiodrilus*—that is to say, the ventral couple are at some little distance from each other, while the lateral couple are very closely approximated.

The clitellum was not very distinctly marked, but appeared to comprise four segments, 14—17.

Dorsal pores could not be detected.

The nephridiopores are placed in front of the lateral setæ.

The oviducal pores, as in all Eudrilidæ, are lateral in position; they are upon the 14th segment, and are quite conspicuous, owing to their being surrounded by a slightly raised margin.

The male generative pore is unpaired and median in position; it lies upon the border-line between Segments 17 and 18: the pore is situated upon the summit of a prominent elevation.

<sup>1</sup> The generic name might be confused with *Heliodrilus* if there were any chance of that problematical form ever being properly identified.

The spermathecal orifice was not visible upon the exterior; by means of transverse sections it was found to be upon Segment 11.

The most characteristic external mark of this species is afforded by a series of sucker-like structures. There are six of these, one to each of Segments 10—15. The last three are accurately median and ventral in position, and are situated on about the middle of their segment. The two in front are placed considerably to the left of the middle line, as shown in fig. 21; the first is nearer to the middle line.

### § Integument.

The epidermis of *Heliodrilus* agrees in every particular with that of *Hyperiodrilus*, as does also the structure of the clitellum. The limits of this modified region of the body-wall could not be ascertained with certainty by an inspection of the worm; transverse sections show that it extends over Segments 14—17.

The peculiar sensory organs which are so characteristic of the Eudrilidæ occur in *Heliodrilus*; their structure calls for no remark, as they resemble in every particular those of *Hyperiodrilus*, which have been already described on p. 236.

The muscular layers of the integument are also identical in every respect with those of *Hyperiodrilus*.

### § Alimentary Tract.

The buccal cavity occupies the first three segments.

The pharynx extends back to the 6th segment.

The œsophagus is very narrow, and passes back without any change as far as the 10th segment; here it becomes narrower, and the lining epithelium is thrown into a series of regular longitudinal rugæ; at the mesentery before the 11th segment, as in the case of the subsequent segments, it becomes a trifle wider, recurring to its former dimensions, which are between one third and one fourth of the diameter of the body of the worm in this region. The first of the two ventral œsophageal pouches lies in this segment, and is directed forwards, having anteriorly no connection with the wall of the œsophagus;



further back it is suspended by two closely approximated and very delicate mesenteries, which later become continuous with a bridge of tissue, along which blood-capillaries pass from the periœsophageal blood-sinus to the vessels of the pouch. This bridge forms the walls of the aperture of communication between the œsophagus and the pouch. The minute structure of the pouch is as in *Hyperiodrilus*, but there is no splitting of the muscular layer of the œsophagus at the origin of the pouch, such as I have figured and described in that earthworm.

The second pouch, which lies in the 12th segment, has a precisely similar origin from the œsophagus, and is identical in all respects with the first.

In the next segment is a third œsophageal pouch, which is very much smaller than either of the other two; its interior is not so subdivided by the development of folds, and the aperture into the œsophagus is distinctly larger; it has the characters rather of a folded-off portion of the œsophageal tube than of a diverticulum.

After this the tube remains narrower for some distance, with the epithelium longitudinally folded; it is here, as throughout its whole extent up to this point, lined by a thin chitinous layer; there is, however, no gizzard upon the œsophagus, and no special thickening of its muscular coat which could be compared to a gizzard.

In the 14th segment the œsophagus becomes wider, and receives the ducts of the calciferous glands; these ducts have exactly the same structure as the œsophagus, and are not of a very greatly inferior calibre. Their epithelium is distinctly ciliated; each duct opens by a wide aperture on to the side of the œsophagus. After the opening of the ducts of the calciferous glands the epithelium of the œsophagus alters its character and becomes ciliated. The diameter of the tube is at the same time larger, and the plexus of blood-vessels more richly developed. Commencing with the 18th segment the alimentary canal is provided with six gizzards, one to the 18th and to each of the five following segments, connected by sections of thin-walled intestine.

### § Body-cavity.

As is usual in earthworms, the first few segments are not separated from each internally by regular septa; irregular fasciculi of muscles attach the buccal cavity and the pharynx to the parietes. The 5th segment is separated from the 6th by the first regular intersegmental septum; this and the 7th which follow are specially thickened, and consist of several layers of muscular fibres. These septa (fig. 48) fit into each other like a series of cups; they arise from points which correspond accurately with the intersegmental furrows.

The cœlom in this worm is further broken up into the compartments which lodge the testes and extremities of the vasa deferentia, and into those which lodge the ovaries and the spermatheca; finally a special sac encloses the supra-intestinal blood-vessel. All these cœlomic chambers are specially described under the different organs which they enclose.

As in other Oligochaeta (cf. Kükenthal 13), the cœlomic corpuscles are of two kinds.

### § Vascular System.

The supra-intestinal vessel exists in *Heliodrilus*, and is connected with the hearts, as Perrier was the first to point out in some other genera.

In *Heliodrilus* this vessel is small, and lies in a special cœlomic space above the gut. A transverse section through the supra-intestinal vessel and this perihæmal sac is illustrated in fig. 46. The sac has exceedingly thin walls, which are connected with the walls of the blood-vessels by irregularly disposed trabeculae; the interspaces are largely filled with corpuscles which have the characters shown in the figure; these cells are quite similar to those which occur in the walls of the intestine. The inclusion of the supra-intestinal vessel in a sac recalls the similar perihæmal space which I have described in *Deinodrilus* (4) as surrounding the dorsal blood-vessel.

The large "hearts" of Segments 11—13 communicate with the supra-intestinal as well as with the dorsal vessel. The

supra-intestinal vessel does not, as it does in *Eudrilus*, become double above each of the ventral œsophageal pouches.

### § Generative Organs.

(1) Female Organs.—As in most of the genera belonging to the family Eudrilidæ, there is a complicated system of cœlomic spaces developed in connection with the ovaries and the other organs belonging to the reproductive system.

The ovaries are paired, and in Segment 13. Each ovary is enclosed in a sac which it almost completely fills; a narrow tube running dorsal to the nerve-cord connects the ovarian sacs of the two sides of the body; there is further a communication between the ovarian sac and the egg-sac of its own side, as in *Teleudrilus* and *Hyperiodrilus*; this communication is effected by a cœlomic tube which is at first very narrow; as it approaches the egg-sacs it becomes wider, and finally forms a somewhat oval sac enclosing the funnel of the oviduct and communicating with the egg-sacs, into which the oviducal funnel also opens. So far as I can make out from a complete series of transverse sections the arrangement is, so far, very like that which has been figured and described by Rosa in *Teleudrilus* (10); but *Heliodrilus* apparently differs from *Teleudrilus*, and certainly agrees with *Hyperiodrilus* in the communication between the right and left ovarian sacs. I found it quite easy to trace the course of the tube which connects the ovarian sac with the considerable space surrounding the funnel of the oviduct; but any doubt as to the reality of this connection is removed in the present instance by the occurrence of ova floating freely in the wide space round the funnel; for the most part these ova were to be observed singly, each surrounded by a follicular layer of flattened cells, of which the nuclei alone were conspicuous: in a few cases the ova were also surrounded or partially surrounded by groups of germinal cells, as a rule comparatively few in number. The ova in the ovary, as well as those which I found in the sinus surrounding the funnel of the oviduct, had a well-developed vitelline membrane, but showed no traces of the

remarkable striated membrane which I shall refer to directly in describing the ova within the egg-sacs.

The oviduct has been incidentally referred to in the foregoing description; it opens into the egg-sac and into the coelomic space continuous with the perigonadial sac; it is a short tube, and passes straight to its opening upon the 14th segment; it is not twisted upon itself, as is the oviduct of *Eudrilus*. The oviduct has fairly thick muscular walls, the fibres of which are for the most part arranged in a series of rings round the tube, and a lining of columnar ciliated cells. The calibre of the oviduct diminishes gradually from the funnel to the external aperture.

The egg-sacs are also situated in the 14th segment: the septum dividing this segment from the one in front is entirely or largely absent; but the position of the egg-sacs within the 14th segment suggests that they lie near to where the anterior wall of that segment should be. The interior of the egg-sacs is divided up by trabeculae anastomosing with each other into a series of very small compartments, only just broad enough to contain a single ripe ovum; the compartments, as in other earthworms, are lined with small peritoneal cells (see fig. 29).

The mature ova do not present any noteworthy differences from those of *Hyperiodrilus*.

*Spermatheca*.—As in *Hyperiodrilus*, there is only a single spermatheca present, which lies on the right side of the body—the opposite side, therefore, to that which the spermatheca occupies in *Hyperiodrilus*. The spermatheca in *Helioidrilus* is a large conspicuous organ, which can be seen, on a dissection of the worm, to reach on to the dorsal side of the gut; it contrasts, therefore, with the very small spermatheca of *Hyperiodrilus*. As in the latter worm, the apparent bulk of the spermatheca is increased by a prolongation of the perigonadial sinus which partially surrounds it; but the arrangement of this sinus in *Helioidrilus* is very curious, and quite unlike that of *Hyperiodrilus*. But before describing the sinus I will direct attention to the characters of the spermatheca itself, which differs in certain points from the



spermatheca of any other Eudrilid, or in fact any other earthworm at present known.

It is a large oval sac lined by columnar cells; a portion of one of the walls is represented highly magnified in fig. 37: below the layer of columnar cells are some smaller cells, the contours of which are not very clear, though their nuclei are; outside these are a few muscular fibres, which make up a layer of no great thickness. The interior of the spermatheca contains a granular substance which appears to be formed by the columnar cells. The calibre of the spermatheca (fig. 41) gradually diminishes towards the apex and towards the ventral side of the body; here the cells lose their glandular character, and become at the same time considerably shorter, so that the muscular coat appears to acquire an additional thickness. The narrow duct of the spermatheca does not open upon the 13th segment as in *Hyperiodrilus*, but bends under the nerve-cord and runs forwards, always lying beneath the nerve-cord, as far as the 11th segment; throughout the whole of its course beneath the nerve-cord it is a narrow tube with thick muscular walls, and a lining of short columnar cells, which, it is perhaps unnecessary to remark, show no traces anywhere of cilia. The diameter of the spermathecal tube in these segments is about equal to that of the nerve-cord. In the 11th segment the spermathecal tube perforates the body-wall, and opens on to the exterior by an inconspicuous orifice which is situated on the median ventral line. The ventral sucker-like organ of this segment is pushed to one side, as shown in fig. 21, and does not therefore interfere with the accurately median position of the spermathecal pore.

The number of segments occupied by the spermatheca is thus considerably in excess of that which is found in any other earthworm.

If the spermatheca is developed in the Eudrilidæ as in the Lumbricidæ by an inpushing of the epidermis, the point of opening will fix the morphological position of the organ; hence *Heliodrilus* serves in this respect to connect the Eudrilidæ with other earthworms, for the spermatheca opens in front of

the ovarian segment, and yet the main part of the organ lies in that segment.

The apex of the spermatheca cannot be seen on a dissection of the worm, for the reason that it lies embedded in a cœlomic sac. Figs. 32—34 represent a series of sections showing the relations of the spermatheca to this sac. The sections are part of a series running from behind forwards; towards the posterior end of Segment 14 the end of the spermatheca is seen to lie between two cœlomic spaces, which are really continuous, and envelop the apex of the spermatheca as seen a few sections later; in this section the extremity of the spermatheca is seen in transverse section to lie in the middle of the cœlomic sac, which is incomplete dorsally: this is, however, merely due to an accidental cut; the sac is really closed. Round the spermatheca is a mass of tissue which is seen in a later section to be the wall of a second sac lying within the first. The spermatheca is pushed against the wall of this, driving it before it for a little way, but it hardly enters the second sac: fig. 34 is therefore a little exaggerated in this particular; the cavity of the spermatheca does not appear to be continuous with that of the second sac, although I should have preferred longitudinal sections to decide the point; in any case the character of the lining cells is absolutely different. This second sac which lies within the first is also closed; it has the same general structure, consisting of two layers of peritoneum, between which are a few fibres of what appears to be muscular tissue; but the lining peritoneum, as shown in fig. 33, is very much thicker, and the cells are larger and rounded.

In both sacs masses of corpuscles lie here and there within the lumen.

The outer cœlomic sac gradually narrows ventrally, and ultimately becomes an extremely narrow tube, which is attached to the spermatheca by the mesentery; it finally becomes continuous with the perigonadial sinus. The general disposition of the female reproductive organs and of these cœlomic sacs connected with them is shown in a semi-diagrammatic form in fig. 41. In

reconstructing this figure from the transverse sections I have put in the intersegmental septa between Segments 12, 13, and 13, 14, which I have not actually observed; I imagine that they will be found to be partially absent, as in *Hyperiodrilus*. The position of the cœlomic sacs is also not quite as in nature; they have been slightly altered to permit of everything being seen in one figure: but these alterations do not affect the mutual connection of the various parts; these are, I trust, accurately displayed in the figure, and also in fig. 36, which represents the apex of the spermatheca (*sp.*). Comparing the arrangement of these parts in *Heliodrillus* with that of *Hyperiodrilus* we find an increase in size of the spermatheca, and a decrease in size of the cœlomic sac involving the spermatheca. The latter, instead of forming a complete ring round the œsophagus completely enclosing the spermatheca on one side, is only developed on one side of the body, and surrounds only the extremity of the spermatheca. The second sac, lying within the dorsal dilated part of the sac which surrounds the extremity of the spermatheca, is peculiar to *Heliodrillus*, and is an extraordinary structure, concerning the meaning or function of which I can offer no suggestion. The enormous development of the lining cells of the circum-œsophageal sacs of *Hyperiodrilus* into the similitude of a glandular epithelium is not found in *Heliodrillus*. It is possible, however, that it is a periodical occurrence which does not happen to have taken place at the time when the only specimen of *Heliodrillus* that I possess was killed. The reduction of the cœlomic sac surrounding the spermatheca not only in size, but also in the extent to which it involves the spermatheca, culminates in *Eudrilus*, where, as far as I can see, there is no vestige of any perispermatic sac left. I should like to make this point quite certain, but in any case it is evident that if such a sac does exist, there must be merely traces of it. The question is whether *Eudrilus* represents the last term in this series of modifications, or whether *Hyperiodrilus* does; from what we know of the anatomy of the *Oligochæta* it seems more reasonable to suppose that the development of these

cœlomic spaces is secondary. Accordingly, as regards the Eudrilidæ, *Eudrilus* represents perhaps the most archaic form. This is also, it may be remarked, in accordance with the fact of its geographical distribution. It occurs in South America, the West Indies, St. Helena, New Caledonia, and New Zealand, being therefore one of the most widely spread of earthworms. It seems, therefore, permissible to argue from *Eudrilus* to the other Eudrilidæ; and this I have attempted to do in the case of the ovarian oviducts and atria (see p. 268). It would be interesting to ascertain how far the spermathecal cœlomic spaces are represented in *Teleudrilus*; the connection between the perigonial sinus and the egg-sac is developed in that genus as in *Heliodrilus* and *Hyperiodrilus*, but not *Eudrilus*.

2. Male Generative Organs.—The testes are, as usual, paired structures which lie in the 10th and 11th segments.

Each testis has a number of processes of unequal sizes, as is so very generally the case with earthworms. The testes, however, do not conform to the general rule in their position; they lie near to the posterior septum of their segment, as in *Acanthodrilus annectens* (4), alone among earthworms at present known.

Furthermore, each testis, instead of lying freely in the cœlom, is surrounded by a small sac, which is only large enough to contain the testis (see fig. 15); this sac is attached to the lateral parietes some way above the nerve-cord by a thin mesentery (fig. 15, *mes.*), and directly to the septum which divides its segment from the one following. This sac has for its size tolerably thick muscular walls, and of course a lining as well as a coating of peritoneal cells.

The vasa deferentia have the same curious arrangement that Rosa was the first to describe (10) in *Teleudrilus*, and which I have already mentioned as occurring in *Hyperiodrilus*; each vas deferens perforates septa 10, 11, or 11, 12, and then, passing back, again perforates them to reach the interior of one of the sperm-sacs which depend from the posterior surface of these septa.



But in *Heliodrilus* there are some differences of detail. The expanded portion of the vas deferens, which lies in front of septa 10, 11, and 11, 12, is proportionately much larger, while the narrow neck by which it is connected with its funnel is much longer than in *Hyperiodrilus*.

Furthermore, the expanded portion lies in the cœlomic space which contains the testis, or rather it is closely invested by a narrow space (figs. 11, 16, and 18, cœlom), which is perfectly continuous with that enclosing the testis. Sections show that there is no real demarcation between these spaces, which form on each side a pair of sacs—one larger enclosing the vas deferens, and one smaller containing the testis. It should be mentioned that the vas deferens itself on the posterior side of each of the septa lies quite freely in the cœlom.

The dilated region of the vas deferens is quite similar in structure to the rest of the tube; it is lined by a layer of low columnar ciliated cells, and is invested by a sheath containing a few muscular fibres. The interior, as in *Eudrilus*, was filled with loosely lying spermatozoa, not compacted together in any way.

The proportions of this dilated sac to the œsophagus are shown in fig. 11, which is copied from a drawing made by the help of the camera lucida. The details of structure and the proportions of the investing sac are more clearly shown in fig. 35.

Atria.—I term the structures in question “atria” rather than “prostates” for the reason that I have given elsewhere (4), and recapitulated briefly on p. 248 of the present paper. They form a pair of long tubes, which were disposed as follows in my specimen. On the right side of the body the atrium, which opens, as already stated, on to the 17th segment, passed straight back as far as the 24th segment; it was then sharply bent back upon itself, and again reached the 17th segment, where it ended blindly. On the left side the atrium was entirely contained within the 17th, 18th, and 19th segments, being much folded (fig. 18).

A dissection of the worm did not show any differentiation of

the atrium into a glandular and an efferent section, such as is found in *Hyperiodrilus* and in other earthworms. There is no such sharp demarcation between the highly glandular more distal part of the atrium and the duct which perforates the body-wall, and is lined with simple columnar epithelium.

The glandular part of the atrium has the typical structure, although the demarcation between the two layers of cells was obscured by the great abundance of secretion; the inner layer could be only detected by the presence of a regular row of nuclei. Towards the external aperture the character of the lining epithelium gradually alters: this alteration affects, in the first place, the thickness of layers; they become gradually thinner, until not far from the external orifice there is but a single layer of cells. The cells in this layer (fig. 39) are of two kinds—large swollen glandular cells with a scanty amount of protoplasm lie embedded in a mass of narrow columnar cells; when the tube enters the body-wall on its way to the exterior the glandular cells disappear, and there are only columnar cells present of a non-glandular character.

The vasa deferentia, as in *Hyperiodrilus* and *Eudrilus*, open into the glandular portion of the atrium, which is, I should remark, not divided into two chambers bound up in a common sheath, as it is in *Eudrilus*. The two vasa deferentia retain their distinctness, and open into the atrium (fig. 42) at some little distance from each other.

The glandular part of the atrium has a very thin muscular sheath; this becomes thicker towards the external orifice, where it is plainly divisible into two layers—an outer layer of circular fibres, and an inner layer of longitudinally running fibres. The layers, however, are even here very thin, and do not consist of more than two rows of fibres. The two atria traverse the body-wall independently of each other, and unite at the bottom of a tubular depression which communicates directly with the exterior. The structure of the parts is such that it does not appear to be capable of eversion as a penis. There are no penial setæ present. The body-wall is considerably thickened in the region of the atria for some distance on

either side of the ventral line; the thickening ceases at the first seta.

A curious fact in relation to the atria is the connection of the nephridia with these organs. In the other segments of the body the nephridia open in front of the lateral setæ; but in the 17th segment the duct of the nephridium traverses the ventral body-wall in an oblique direction, and joins the atrial duct just before its opening into the external depression already spoken of (fig. 40). As this occurred on both sides of the body it does not seem likely to be an abnormal condition characterising the individual only.

§ Summary of more important facts in the structure of *Heliodrilus* and *Hyperiodrilus*.

(1) The epidermis is furnished as in other Eudrilidæ, and in that family alone, with peculiar organs possibly of a sensory nature; these consist of a central nucleated core surrounded by many nucleated sheaths; the organs have a certain resemblance to Pacinian bodies of Vertebrates, and are scattered irregularly over the surface of all the segments save the first.

(2) The alimentary canal has a single pair of large lobed calciferous glands in the 13th or 14th segment; in each of Segments 10, 11, 12, is a median diverticulum of the œsophagus, of which the epithelium is much folded, so that it presents the appearance of a series of parallel tubes; peripherally the cells themselves are excavated, and form a ramifying system of ductules; the pouch of Segment 10 is smaller, and the foldings are much simpler and do not anastomose. There is no anterior gizzard, but six gizzards, one to each segment, at the junction of the œsophagus with the intestine.

(3) The supra-intestinal blood-vessel in the œsophageal region is enclosed in a special cœlomic compartment, which is almost filled by deeply staining nucleated corpuscles.

(4) The male genital pore is single and median upon Segment 17. The "atria" are glandular and very long; in *Heliodrilus* the vasa deferentia open into them in both genera. In neither are there any penial setæ, but in *Hyper-*

riodrillus there is a penis, which is a hollow process of the body-wall. In both genera the vasa deferentia open in the 11th and 12th segments into the interior of the sperm-sacs; each vas deferens perforates the septum, from which the sperm-sac depends, twice.

(5) The ovaries are enclosed in special cœlomic sacs which communicate with the egg-sac, and are prolonged dorsally so as to entirely (*Hyperiodrilus*) or partially (*Heliodrilus*) enclose the single spermatheca which opens on the middle line of the 13th (*Hyperiodrilus*) or 11th (*Heliodrilus*) segment; in the latter case the spermatheca itself lies in the 13th segment, and has a long duct. In *Hyperiodrilus* the perigonadial sacs form a ring round the œsophagus, and are connected with a dorsal unpaired sac.

### III.—Some Notes upon *Nemertodrilus griseus*, Mich.

The principal points in the anatomy of this Eudrilid have been already made out by Michaelsen; there are, however, a few facts of minor importance which I have been able to note down from the examination of specimens which Dr. Michaelsen was so good as to place at my disposal. With regard to the female reproductive organs, I can only confirm the accurate description given by Michaelsen; but the structure of these parts suggests certain reflections concerning the homologies of the various organs which constitute the female reproductive system in the Eudrilidæ. As Michaelsen has stated, the cavity of Segment 13 is greatly reduced, so that the ovaries are enclosed in a narrow chamber; the receptaculum ovarum communicates with this segment, and is also connected with a large pouch extending on each side of the body through several segments.

Michaelsen suggests that these large pouches may be possibly the equivalents of the receptacula and the distal part of the spermatheca. The proximal part of the spermatheca on this supposition is represented by a pair of orifices, of which more later, opening into the exterior of the body from the reduced cavity of the 13th segment. Dr. Michaelsen decides, however,



that most probably the sacs in question are simply extensions of the egg-sacs, inasmuch as there is no definite break between them and the egg-sacs; the trabeculæ which divide up the interior of the egg-sac into a series of more or less isolated compartments, as in other earthworms, get gradually less and less until the cavity becomes perfectly smooth.

The paired orifices upon the 13th segment are considered by Michaelsen to represent the rudiments of spermathecæ which open into this segment in other Eudrilidæ.

These orifices are fringed with numerous frayed-out cellular processes, which would appear to be of the nature of proliferations of the peritoneum.

The effect of these must be analogous to the twigs of a lobster-trap; they would prevent egress from the interior of the segment, but would permit the penis to be thrust in. It seems most likely that these orifices are used in copulation; the sperm can then readily find its way into the interior of the egg-sacs; and as a matter of fact I can fully confirm Michaelsen's statement that bundles of spermatozoa are found in the interior of these sacs.

The large sacs which extend from the 14th to the 17th segment seem to me to be in all probability equivalent to the cœlomic sacs which I have just described in *Hyperiodrilus* as encircling the œsophagus and fusing above it to form a large unpaired sac. I consider that Michaelsen is quite right in deciding that they do not represent a portion of the two spermathecæ cut off from the duct leading to the exterior; but, on the other hand, I regard my own identification of these openings as a little more probable than that of Michaelsen.

The relationship of the egg-sacs to the cœlomic sacs in this genus and in *Hyperiodrilus* is something like that of the sperm-sacs in *Dichogaster* and other worms to certain sacs connected with the testes and the funnels of the vasa deferentia. It is, perhaps, worth remarking that the connection of these sacs above the intestine is curiously paralleled by the connection of the ovarian sacs in *Hyperiodrilus*. Next, with regard to

the paired orifices upon Segment 13, Michaelsen considers these to be the remains of the spermathecæ which open on to this segment in *Eudriloides*, *Teleudrilus*, and other *Eudrilids*. This identification cannot be regarded at present as being anything more than possible. The spermathecæ of *Lumbricus* are developed as involutions of the epiblast, and if the course of development of the spermathecæ in the *Eudrilidæ* is the same, it is not likely that they could ever come to be represented by pores.

Considering the matter necessarily in the absence of any knowledge of the development of the parts in question, it seems possible to regard these pores as the rudimentary equivalents of oviducts.

In describing the structure of *Eudrilus* I have pointed out that there are apparently two pairs of oviducts present in that worm. One pair, represented in all other earthworms, open on to the 14th segment; the other pair are short tubes connected with the sac involving the ovary in Segment 13; they open in common with the spermatheca and the other oviducts on to the 14th segment.

These peculiarly modified organs in *Nemertodrilus* are quite intelligible on the hypothesis that they have been derived from the corresponding organs of *Eudrilus*.

I pointed out that there was some evidence in favour of regarding the oviducts of Segment 13 as being in course of degeneration; they are very short, with feebly developed walls, and the lining epithelium is not ciliated. This reduction is carried further in *Nemertodrilus*; the oviducts are reduced to the condition of the oviducts in the *Enchytraeidæ*, where there is little more than a pair of orifices. At the same time the cœlomic cavity of the segment is greatly reduced; this renders it easy for the ova to reach the exterior through the oviducts of the 14th segment, which are apparently as well developed as in *Eudrilus*.

In *Teleudrilus* there is no trace (?) of the oviduct of Segment 13, and, except for the continuity of ovarian sac with the receptaculum, the female reproductive organs of this genus

are not far removed from those of the more typical Oligochaeta.

It is perhaps possible to regard the pores in *Nemertodrilus* as the earlier condition. Observations upon the abdominal pores and oviducts of certain Ganoids and Teleosteans seemed at one time to indicate that the pores were the primitive structures, and that a groove in the peritoneum, converted later into a tube, connected these pores with their respective gonad. But if Jungersen<sup>1</sup> is right in looking upon the oviducts of Teleostei as being after all Müllerian ducts, this view must fall to the ground. Sedgwick<sup>2</sup> brings forward many facts towards proving that in *Peripatus* the genital ducts are coelomic sacs communicating with the exterior by pores. The ovary in *Nemertodrilus*, closely invested by the coelom which opens on to the exterior by a pore, is comparable to the gonad and its duct in *Peripatus*; it is possible, as I have already suggested, that in *Eudrilus* the oviduct is only a portion of the coelom connected with a pore; but I am more disposed to hold that *Nemertodrilus* is in these points a degenerate form of *Eudrilus*; the reduction of the coelom of Segment 13, and the disappearance of the spermathecae bears out this view.

In any case two pairs of oviducts seem to imply two pairs of ovaries, which are reduced to one by the disappearance of the anterior oviducts. And we thus arrive at the normal condition of the female reproductive organs in earthworms.

In *Teleudrilus* the complete disappearance of one pair of oviducts is correlated with the disappearance of the second pair of ovaries.

In short, the new facts discovered by Dr. Michaelsen lend support to the conclusions which I formulated in the paper referred to above, apart altogether from the question as to the

<sup>1</sup> "Beiträge zur Kenntniss der Entwicklung der geschlechtsorgane bei den Knochenfischen," 'Arb. Zool. Zoot. Inst.,' Würzburg, 1890.

<sup>2</sup> "A Monograph of the Development of *Peripatus capensis*," 'Studies Morph. Lab. Cambridge,' vol. iv, part 1.

primitive or non-primitive condition of the reproductive system in *Eudrilus*.

The atria present the appearance of the corresponding organs in *Acanthodrilus* or *Pontodrilus*—that is to say, they form two somewhat bent tubes of an opaque white colour; they differ, however, in the fact that it is impossible to distinguish a muscular and a glandular portion: in the two genera mentioned, and in many other forms in which the atria are tubular, the organ communicates with the exterior by a narrow duct; this duct is lined with an epithelium which is not in the least glandular, and is surrounded by a tolerably thick muscular coat. In *Nemertodrilus* no such duct is present; the organ is identical in structure throughout with the glandular part in *Acanthodrilus*: its epithelium is of two kinds; the innermost layer is formed by a single row of unusually short columnar cells; beneath these are the usual layers of pyriform gland-cells, each with a long slender prolongation which reaches, or nearly reaches, the lumen of the gland.

As Perrier (8) first remarked, the atrium of *Eudrilus* is remarkable on account of its nacreous appearance and perfectly straight course. The nacreous appearance is due to an enormously thick muscular coat, which I figured in transverse section in a paper dealing with the structure of *Eudrilus sylvicola* (1).

In *Nemertodrilus*, as I have already implied by comparing the appearance of the organs to that presented by the atria of *Acanthodrilus* and *Pontodrilus*, the nacreous appearance is entirely wanting. Sections of the atrium, however, show that the muscular coat itself is not absent, but is greatly reduced as compared with *Eudrilus* and, according to Rosa's observations, *Teleudrilus*. The whole organ, in fact, is a little more degenerate than that of *Eudrilus*. Considering the absence of the duct, which is so universal a feature of the atrium among earthworms, I should be disposed to regard the atrium of *Nemertodrilus* as having been derived from that of *Eudrilus* by reduction,



and not vice versâ. There is at any rate nothing in the facts which is opposed to this view, though the converse might be asserted with some probability.

As Dr. Michaelsen has pointed out, the two vasa deferentia of each side retain their distinctness, but are accurately superimposed, thus giving rise to the impression that but one tube is present.

A curious peculiarity in the vasa deferentia of *Eudrilus*, which appears to be confined to that genus and to other *Eudrilidæ*, was first pointed out by myself. In those genera each vas deferens has, like the atrium into which it opens, a well-developed muscular tunic; each vas deferens, moreover, in those very aberrant earthworms commences with a very wide dilatation immediately connected with the funnels.

In both these points *Nemertodrilus* differs from its two allies—the vas deferens has neither the oval or spherical dilatation nor the muscular coat; it conforms, in fact, in every particular to the usual type met with among earthworms.

Michaelsen mentions that the two vasa deferentia of each side, maintaining their distinctness to the very last, become lost in the body-wall just in front of the atria.

This is undoubtedly the impression which a dissection of the Annelid produces, but it is not perfectly accurate. The point is one of some little importance as touching the affinities of *Nemertodrilus* to *Eudrilus*.

In the latter genus I showed that the vasa deferentia opened into the atrium at about the middle of its length. In *Teleudrilus* Rosa has stated that the vasa deferentia also open into the atrium.

In *Nemertodrilus* a series of longitudinal sections shows that the two vasa deferentia cross the atrium close to its external aperture; they then traverse the muscular coat exactly as in *Eudrilus*, and each may be recognised still preserving its independence as a ciliated tube lying between the epithelial lining and the muscular coat. They finally open into the interior of the atrium.

Michaelsen has pointed out two characters in which *Nemer-*

todrillus differs from the Eudrilidæ: the first is the position of the nephridiopores, which are situated in front of the ventral instead of the lateral setæ; the second character is the absence of any glandular diverticula to the alimentary tract.

To these I can add a third character—the absence of those integumental bodies which occur in all other Eudrilidæ that have been sufficiently well examined.

The structure of *Nemertodrillus* shows that it is decidedly an aberrant member of the family Eudrilidæ. But the alterations in structure from the typical Eudrilidæ do not definitely point in the direction of the Cryptodrillidæ, with which family, as I distinguish it, Rosa unites the Eudrilidæ.

The only reasons for referring this genus to the Eudrilidæ are (1) the absence of spermathecæ lying in front of the testes, (2) the completely separate vasa deferentia opening into the interior of the atrium, (3) the large cœlomic sacs connected with the egg-sacs, (4) the muscular oviduct.

The absence of any specialisation into the atrium, of dilatation upon the vasa deferentia, of integumental organs, are perhaps indications of degeneration. The fact that the ovary is not, as is the rule among the Eudrilidæ, enclosed in a special compartment of the cœlom, might be used as an argument for the primitive position of *Nemertodrillus* among the Eudrilidæ were it not for the reduction of the cavity of the thirteenth segment. The reduction of this segment renders the development of any such sacs unnecessary, though of course it does not necessarily follow that they were originally present and have been lost. On the other hand, the pores upon Segment 13, whether Michaelsen's explanation of them or mine be correct, seem to be in all probability rudimentary structures of some kind.

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## LIST OF MEMOIRS REFERRED TO.

1. BEDDARD, F. E.—“Contributions to the Anatomy of Earthworms.”  
I. “On the Structure of *Eudrilus sylvicola*,” ‘Proc. Zool. Soc.,’  
1887, p. 372.
2. BEDDARD, F. E.—“Note on the Structure and Development of the  
Ovum in an Annelid (*Eudrilus*),” ‘Journ. Anat. and Phys.,’ October,  
1887, p. 9.
3. BEDDARD, F. E.—“Contributions to the Anatomy of Earthworms, with  
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## EXPLANATION OF PLATES XVI—XX,

Illustrating Mr. Frank E. Beddard's memoir "On the Structure of Two New Genera of Earthworms belonging to the Eudrilidæ, and some Remarks on Nemertodrilus."

## PLATE XVI.

*Hyperiodrilus africanus.*

FIG. 1.—Female reproductive apparatus, seen after opening body-wall from above and removing one of the calciferous glands. *ca.* Calciferous gland. *sp'*. Large cœlomic pouch, connected with a periœsophageal ring. *r. o.* Receptaculum ovorum. *od.* Oviduct. *œs.* Œsophagus. *d. v.* Dorsal vessel. *s.* Intersegmental septum.

FIG. 2.—Integumental sense (?) organs in longitudinal section.

FIG. 3.—The same in situ. Transverse section. This drawing also shows the lymph-spaces between the fibres of the circular muscular coat.

FIG. 4.—Diagram of structure of the same.

FIG. 5.—Diagrammatic lateral view of the different parts of the female reproductive apparatus. The segments are numbered. *sp.* Spermatheca. *ov.* Ovary. Other letters as in Fig. 1. The walls of the cœlomic spaces are here and there cut away so as to display the contained viscera.

FIG. 6.—Anterior segments from above, to show form of prostomium.

FIG. 7.—A portion of integument, highly magnified, to show relationship of nephridiopore (*np.*) to seta (*s.*) of dorsal pair.

FIG. 8.—Female reproductive apparatus removed from body, but with its relations to other organs indicated. *β.* Bursa. Other letters as in Figs. 1 and 5.

FIG. 9.—Transverse section to show arrangement of setæ.

FIG. 10.—Transverse section through a portion of perigonadial sac and the neighbouring body-wall. *n.* Nephridium. Other lettering as in Fig. 8.

FIG. 11.—Diagrammatic view of female reproductive organs. The periœsophageal sinuses and the unpaired dorsal sac in which they meet are pushed back; the œsophagus is cut away, but the nerve-cord is left. On the left side the anterior wall of perigonadial sinus and periœsophageal sinus is removed in order to display enclosed viscera. The segments are numbered. Lettering as in Figs. 1, 5, and 8.

FIG. 12.—A few segments pressed out to show arrangement of setæ (*s.*) and position of nephridiopores (*n.*) by lateral setæ.



## PLATE XVII.

*Hyperiodrilus africanus* and *Heliodrilus lagosensis*.

FIG. 13.—Diagrammatic transverse section of *Hyperiodrilus* in neighbourhood of the female reproductive organs, to be compared with

FIG. 14, representing a corresponding section through *Heliodrilus*.

FIG. 15.—*Heliodrilus*. The sac, containing testis and its attachment to the body-wall. *mes.* Mesentery. *lm.* Longitudinal muscle-fibres.

FIG. 16.—*Heliodrilus*. Dissection of cœlomic space, containing testis and first part of vas deferens. The wall of the sperm-sac is partly removed to show funnel.

FIG. 17.—Transverse section through alimentary canal (*int.*) and the dilated part of the vas deferens (*v. d.*) of *Heliodrilus*.

FIG. 18.—Dissection to show male reproductive system of *Heliodrilus*. *ves. sem.* Sperm-sacs. *atr.* Atrium uncoiled; on the opposite side it is left coiled up.

## PLATE XVIII.

*Hyperiodrilus africanus* and *Heliodrilus lagosensis*.

FIG. 19.—*Hyperiodrilus*. Magnified view of the ventral surface of a portion of Segment 17 to show the papillæ (*pap.*) connected by grooves with the penis. The left papilla is on the boundary line between Segments 16 and 17; the right hand one in the latter segment. *s.* Setæ.

FIG. 20.—17th and neighbouring segments of another individual.

FIG. 21.—*Heliodrilus*. Ventral aspect of anterior segments to show position of male pore (♂), spermathecal pore (♀), and papillæ (*pap.*).

FIG. 22.—*Hyperiodrilus*. A third individual, 17th and neighbouring segments. In this specimen the papillæ are symmetrical and upon the 17th segment.

FIG. 23.—*Hyperiodrilus*. Lateral view of genital segments, showing oviducal pores (♀), nephridiopores (*n.*), and male pore (♂).

FIG. 24.—*Hyperiodrilus*. Ventral view of 17th and neighbouring segments of same individual as that represented in Fig. 19. *p.* Penis, slightly protruding from male pore. ♀ Spermathecal pore. *pap.* Papillæ.

FIGS. 25 and 26.—*Hyperiodrilus*. Sections through ventral œsophageal pouch at two points; for explanation see text.

FIG. 27.—*Hyperiodrilus*. Vasa deferentia, with their peritoneal coat.

FIG. 28.—*Hyperiodrilus*. Dilated portion of vas deferens.

FIG. 29.—*Heliodrilus*. A portion of a section through the receptaculum

ovorum. *ov.* Mature ovum with membrane. *germ.* Germinal cells attached to ovum.

FIG. 30.—Hyperiodrilus. Transverse section through unpaired œsophageal pouch, to show blood-spaces (*black*) and lumina between folds of epithelium.

FIG. 31.—Hyperiodrilus. Origin of unpaired œsophageal pouch (*ca.*). *d.* Glandular (?) cells found among the ordinary epithelial cells. *p.* Peritoneum.

## PLATE XIX.

### *Heliodrilus lagosensis.*

FIGS. 32—34.—A series of sections to show the investment of the apex of the spermatheca by cœlomic spaces.

FIG. 32. The apex of the spermatheca (*sperm.*) lies between two out-growths of the cœlomic sac (*cœl.*).

FIG. 33. The apex of spermatheca (*sp.*) is now enclosed within the cœlomic sac, and the commencement of a second cœlomic space within the first is just visible.

FIG. 34. This section lies beyond the apex of spermatheca, which just reaches the interior of the sac (*cœl'*.), lying within that (*cœlom*) which is continuous with the perigonadal sac.

FIG. 35.—Diagrammatic representation of the extremity of the spermatheca (*sp.*) enclosed within a sac, which is itself enclosed within an extension of the perigonadal sac; the narrow canal leading from the latter to the ovary is seen. Portions of the walls of the different sacs are removed to show their contents.

FIG. 36.—Section through dilated portion of vas deferens with surrounding cœlomic sac. *vd. e.* Ciliated epithelium of vas deferens. *p.* Peritoneum covering cœlomic sacs; in its interior are seen clumps of cells.

FIG. 37.—Section showing structure of spermatheca. *ep.* Epithelium. *gr.* Granular matter thrown off from cells into the interior of the spermatheca. *m.* Muscular coat with blood-vessels, shown black.

FIG. 38.—Section showing structure of distal part of atrium. *musc.* Muscular coat. *n.* Nuclei of innermost epithelial layer.

FIG. 39.—Section showing structure of atrium, near to its external orifice. Large glandular cells (*gl.*) are seen, separated by interstitial cells. *musc.* Muscular coat.

FIG. 40.—Section through common orifice of atrium and nephridium.

FIG. 41.—Diagram of female reproductive system. *sperm.* Spermatheca. *sp. o.* Its external orifice. *ov.* Ovarian sac, represented as being cut open on

the left side to show the contained ovary. *sp. sac.* Cœlomic sac involving a second sac, in which lies apex of spermatheca; the walls are represented as partly cut away. This part is shown more highly magnified in Fig. 35. *r. o.* Receptaculum ovarum, represented as being cut open on left side to show funnel of oviduct (*od.*). The nerve-cord is removed for the greater part to show the underlying narrow duct of the spermatheca.

FIG. 42.—Section through atrium at the point where the two vasa deferentia open.

FIG. 43.—Section through a portion of one of the ventral œsophageal pouches. *bl.* Blood-vessels, coloured black. *sh.* Peritoneal covering. *n.* Intra-cellular part of lumen.

## PLATE XX.

*Hyperiodrilus africanus*, *Heliodrilus lagosensis*, *Nemertodrilus griseus*, *Teleudrilus ragazzii*, and *Eudrilus*.

FIG. 44.—*Hyperiodrilus*. Diagram of male reproductive organs. *s. c.* Sperm-sac. *v. d.* Vas deferens. Atria and testes also shown.

FIG. 45.—*Hyperiodrilus*. Longitudinal section through duct, leading from egg-sac. *c.* Outer. *c'.* Inner peritoneum.

FIG. 46.—*Hyperiodrilus*. Supra-intestinal vessel, enclosed within a peritoneal space, which is divided by trabeculæ. In the interstices lie clumps of cells. The perihæmal sac is connected to the dorsal vessel (*d. vess.*) by a mesentery.

FIG. 47.—*Hyperiodrilus*. Dissection of the anterior end of the worm. *sp'.* Anterior. *sp.* Posterior of thickened intersegmental septa. *œs. sac.* Œsophageal pouches. *ca.* Calciferous glands. *sp.* Cœlomic sac, connected with pericœsophageal ring, seen anteriorly. *v. d.* Vas deferens. *at.* Atria. *g.* First of posteriorly-situated gizzards. The segments are numbered from the 6th to the 17th.

FIG. 48.—*Heliodrilus*. A few of anterior segments to show overlapping and interconnection of thickened intersegmental septa.

FIG. 49.—*Hyperiodrilus*. Ripe (*b*) and nearly ripe (*a*) ovum. *z. r.* Zona radiata. *v.* Vitelline membrane.

FIG. 50. *Hyperiodrilus*. Germinal cells from ovary.

FIG. 51.—*Hyperiodrilus*. Section through ovarian sacs, to show their interconnection. *v. b.* Ventral blood-vessel.

FIG. 52.—*Heliodrilus*. Gizzards and commencement of intestine.

FIG. 53.—*Eudrilus*. Diagram of female reproductive system. *ov.* Ovary, surrounded by a sac continuous with a duct (*od.*). *ov'.* Second ovary, sur-

rounded by a sac (receptaculum ovarum) continuous with oviduct (*od'*). *sp.* Spermatheca. ♀. Female pore.

FIG. 54.—*Nemertodrilus*. A similar diagram. Lettering as above. *od.* is simply a pore.

FIG. 55.—*Teleudrilus*. A similar diagram. Letters as above.

FIG. 56.—*Hyperiodrilus*. Terminal portion of atria, and their connection with penis. *v. d.* Vas deferens. *pr.* Glandular part of atrium. *m.* Muscular part of ditto. *p.* Penis.



## The Renal Organs of Certain Decapod Crustacea.

By

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With Plates XXI & XXII.

IN a paper published during the summer of 1889,<sup>1</sup> I gave an account of the excretory apparatus of *Palæmon serratus*, in which I drew attention to the enormous development of the bladder, which extends dorsally from the level of the green glands to the anterior boundary of the pericardium.

The existence of a well-developed nephro-peritoneal sac, having many of the relations of the "cœlomic" body-cavity of other types, had not, at the time of publication of my paper, been recognised in any Crustacean. I have since observed the occurrence of a similar sac in a large number of Decapoda; and M. P. Marchal,<sup>2</sup> who has evidently worked without any knowledge of my observations, has described similar structures in a considerable number of genera.

The object of the present communication is to give an account of the renal organs of certain Carididæ (*Pandalus*, *Virbius*, and *Crangon*) in which the structure of the green gland itself is modified in a very remarkable manner.

Before proceeding to a description of the genera which it is proposed more particularly to consider, it may be well to give

<sup>1</sup> 'Journal of the Marine Biological Association,' N.S., vol. i, p. 162.

<sup>2</sup> 'Comptes Rendus,' cxi, 12, and cxi, 16.

a short summary of the results already arrived at concerning the structure of the excretory apparatus in the Prawn.

The nephro-peritoneal sac of *Palæmon* is a median, unpaired structure, lying in the cephalothorax, dorsal to the alimentary canal, and ventral to the ophthalmic artery and to the median dorsal blood-sinus. Its walls are composed of a single layer of flattened excretory epithelium, which has the power of absorbing indigo carmine and similar substances when these are injected into the blood of the living animal. Posteriorly the nephro-peritoneal sac is in close contact with the anterior extremity of the generative gland; anteriorly it gives off on each side a narrow tube, which passes vertically downwards beside the œsophagus, and, after passing under the œsophageal nerve-commissure, bends outwards to open into the urinary bladder of its own side. The ducts of opposite sides communicate with one another, not only dorsally, through the cavity of the nephro-peritoneal sac, but also vertically, by means of a transverse commissure which passes in front of the œsophagus, and bears a conspicuous dilatation in the cavity of the upper lip.

A diagram of the whole arrangement is given in Pl. XXII, fig. 9; the relations to the other systems of organs, as seen in transverse section, may be gathered from figs. 1 and 2.

The bladder, besides receiving the openings of the nephro-peritoneal ducts, gives off on the one hand the ureter, and on the other the system of excretory tubules of the green gland. These last form a complex mass of branching tubules, which are in close contact one with another, and which form the glandular substance of the green gland. In my former paper I pointed out the existence, in this glandular plexus, of several excretory tubules, an observation confirmed by M. Marchal. I was unable last year, and have still been unable, to convince myself that the various tubules anastomose freely with one another in the way described by this observer. Be this as it may, however, the tubules, after a tortuous passage through the substance of the green gland, unite to open by a common aperture into the "end-sac," whose structure has been re-

peatedly described, especially by Grobben, Marchal, and myself.

In my former paper I described only the arrangement found in *P. serratus*, and the observations of M. Marchal, which are in complete agreement with my own, relate also to this species. I have since satisfied myself that the excretory system of *P. squilla*, and that of *Palæmonetes varians*, are practically identical with that of the species described.

In the genera *Virbius*, *Pandalus*, and *Crangon*, a series of modifications may be observed, resulting in the disappearance of the whole tubular portion of the green gland, and the hypertrophy and specialisation of the end-sac. The nephro-peritoneal sacs are also arranged in a manner strikingly different from that which obtains in the Prawns.

The nephro-peritoneal sacs are nearly identical in arrangement in the three genera in question. Their relations will be readily understood from the diagram, fig. 10, and the transverse section of *Pandalus brevirostris*, fig. 3. The bladder gives off from its internal aspect a duct, which is both wider and shorter than the corresponding duct of *Palæmon*, the duct from each bladder passing inwards under the œsophageal commissure of its own side, and opening into a large sac, which does not communicate with its fellow of the opposite side, and which does not extend to the middle dorsal line above the alimentary canal. The median wall of each sac is closely applied partly to the lateral and ventral wall of the stomach, and partly to the median face of the sac of the opposite side, so that the stomach appears in section to be supported by a well-marked ventral mesentery, which exists beneath the part of that organ which projects in front of the œsophagus, as well as beneath its post-oral region. Both bladder and sac give off numerous processes, which ramify in the base of the antennæ and among the organs of the thorax. The epithelium forming the walls of these organs is in all cases striated and excretory, being very closely similar to the corresponding epithelium of the Prawns. In none of

the three genera is there any connection between the wall of the nephro-peritoneal sacs and the generative glands.

The structure of the "green gland" differs in each of the three genera.

In *Virbius* (varians) a horizontal section through the bladder and "green gland" has the appearance represented in fig. 4. The bladder itself is bounded by a single layer of epithelial cells, whose inner margins frequently project irregularly into the cavity of the organ. These cells exhibit, especially in their peripheral portions, the well-marked longitudinal striation which is so constantly noticed in excretory tissues; their internal portions are, however, frequently vacuolated, and their free internal borders are ragged and indefinite. When this condition prevails the cavity of the bladder is seen (in stained sections) to contain an irregular, granular coagulum, which absorbs hæmatoxylin with readiness. The nuclei of the bladder-cells are oval, and of moderate size; they stain deeply, and do not, in preparations preserved in corrosive sublimate, exhibit any very evident reticulum. The cells composing that portion of the wall of the bladder which invests the end-sac are flatter and more regular than the others; they stain more deeply with hæmatoxylin, and the longitudinal striation is perhaps more evident in these than in the other bladder-cells.

The renal tubule is single, and has a much wider lumen than any of the corresponding tubules of *Palæmon*. It leaves the bladder at the postero-external margin of that organ, and is at first directed nearly horizontally outwards. After passing outwards for a very short distance, however, the tubule turns backwards and then inwards, so that it becomes U-shaped, and opens into the end-sac. The general direction of the single renal tube is that just described, but it does not lie in one plane with such accuracy as to enable it to be included in a single section. In the section figured (Pl. XXI, fig. 4) the two ends of the tube only are seen, the one leaving the bladder, the other entering the end-sac. By examination of the following sections, the course of the tube was determined



as that shown by the dotted lines in the figure. [The determination of such a point is so easy that it has not seemed worth while to publish the figures of the sections upon which it rests.]

It is evident that the single, wide, U-shaped tube, running from the bladder to the end-sac, is the only representative in *Virbius* of the complicated plexus which goes to make up the mass of the green gland in the Prawn. In structure, its walls somewhat resemble those of the bladder already described. They are, however, higher and more regular, their internal extremities of the component cells being less given to the exhibition of vacuoles and irregular processes. The longitudinal striation is more evident, and the nuclei are larger and stain more deeply with hæmatoxylin. Before opening into the end-sac, the lumen of the renal tubule contracts considerably, so that the orifice, by which the cavities of the two structures communicate, is very small. Owing to its size and position, it is exceedingly difficult to demonstrate this opening in transverse sections, but in carefully adjusted horizontal longitudinal sections it is, as will be evident from the figure, easily to be seen.

The end-sac itself is completely enveloped by a layer of bladder epithelium, the wall of the bladder being invaginated by it. The epithelium of the bladder is, however, not in absolute contact with that of the end-sac, the two being separated by a blood-space (left white in the figure). I have not been able to detect any epithelium bounding this blood-space; and I am inclined to believe, after careful examination, that no such epithelium exists. The account given by Grobben of the end-sac of *Palæmon treillianus* leads to the belief that the renal vessels of this species end in lacunæ which are devoid of epithelial lining; and I have been unable to demonstrate an epithelium in the smaller blood-spaces of the kidney of *Pandalus (annulicornis and brevirostris)*. Professors Claus<sup>1</sup> and Lankester<sup>2</sup> have, as is well known, arrived inde-

<sup>1</sup> 'Arb. Zool. Inst. Wien,' Bd. v, Heft 3 (1884).

<sup>2</sup> This Journal, vol. xxv, p. 518.

pendently at the conclusion that the blood system of the Decapod Crustacea is everywhere closed; but I am not aware that either of these observers has paid special attention to the blood-supply of the renal organs. Nevertheless, the positive testimony of two such accomplished anatomists makes me hesitate to lay undue stress upon my own failure to demonstrate an epithelial lining to the particular spaces under consideration.

The epithelium of the end-sac, like that of the rest of the excretory system, is everywhere one cell thick. The individual cells stain more deeply than do those of the bladder and renal tubule; their protoplasm is crowded with granules, which are, however, not arranged in regular rows, so that the cells do not exhibit a longitudinal striation. The nuclei stain very deeply, and exhibit, in specimens preserved in corrosive sublimate, one or two large nucleoli, with no recognisable trace of a chromatin reticulum. The inner extremities of these cells are much vacuolated, and are very irregular; the vacuoles frequently containing spherical concretions of a homogeneous material, which stains slightly with hæmatoxylin, less readily with borax carmine. The cavity of the end-sac is generally found to contain a greater or less quantity of granular clotted material, which appears in sections as a deeply staining, finely granular reticulum.

In *Pandalus* (*annulicornis* and *brevirostris*) a further deviation from the tubular type of nephridium occurs, the renal tubule being in the adult condition entirely absent, while the whole body of the "green gland" is built up of the curiously modified end-sac and the associated portion of the wall of the bladder.

I have not followed the early phenomena of the development of the kidney; but in the late "Mysis" stage the relations of kidney and bladder present a striking resemblance to those which are permanent throughout life in *Virbius*. The appearance presented at this stage in horizontal longitudinal section is shown in Pl. XXI, fig. 5.

The bladder is large compared with the size of the renal

tubule and end-sac ; its cells are, for the most part, pale and uniformly granular, having no trace of longitudinal striation ; their nuclei are rounded, and do not stain deeply. At this stage there is little difference in appearance between that portion of the bladder-wall which is immediately adjacent to the end-sac and the remainder. The cells adjoining the end-sac are slightly smaller than the rest, and their margins slightly more regular, but in other ways the characters of the bladder-cells are everywhere the same ; and the sac, though closely applied to the wall of the bladder, causes hardly any invagination of that structure.

The renal tubule (Pl. XXI, fig. 5) is very short, being simply represented by the curved neck which connects the end-sac with the bladder. Its walls are very similar in structure to those of the bladder itself, and its lumen is very narrow.

The end-sac (Pl. XXI, fig. 5) is small, and bounded by finely granular or nearly homogeneous cells, the nuclei of which are clear and vesicular in appearance. Between the end-sac and the wall of the bladder is a lacunar blood-space ; and both here and on the outer side of the end-sac are groups of connective-tissue cells.

In a very young *Pandalus annulicornis*, which had apparently only just acquired the adult characters, the appearance presented by a transverse section through the excretory organs is shown in fig. 6. [I have only obtained a single individual of this age, and before the present investigation was commenced I had prepared transverse sections of the specimen. I have therefore been unable to figure a section in the same plane as those above described.]

The wall of the bladder exhibits already a distinct specialisation into two regions, one lying beside the end-sac, the other having no relation with that organ. In the latter portion, which of course includes the greater part of the bladder, the cells exhibit distinct indications of longitudinal striation in their peripheral parts, and their inner extremities are often vacuolated. The portion which adjoins the end-sac consists of cells which are more columnar than those of the general



surface of the bladder, which stain more deeply, and are more crowded with granules. The nuclei of these cells exhibit frequent indications of division.

The end-sac is by this time more closely applied to the bladder, the wall of which it invaginates to such an extent that about half its surface is invested by a layer of bladder-cells. The epithelium of the end-sac is made up on the side next the bladder of small cubical cells, and on the opposite side of cells which are somewhat flattened. The protoplasm of all these cells is pale and coarsely granular, as it remains throughout life. The connective tissue, which was noticed in the last stage, has slightly increased in amount, and the blood-spaces between end-sac and bladder are much better developed than during the "Mysis" stage. The renal tubule has ceased to be distinguishable as a separate region, and the end-sac now opens by its posterior extremity directly into the bladder.

The stages in the further development of the bladder I have been unable to observe, my remaining material consisting entirely of adult individuals. It is evident, however, that the end-sac becomes completely surrounded by the wall of the bladder, so that it finally projects freely into the cavity of that organ, being attached to the bladder-wall by a narrow stalk, on which is situated the communication between the cavities of the two structures.

While the process of enclosure of the end-sac by the bladder is going on, the wall of the first-named organ becomes produced into a number of complicated branched papillæ, which project into the cavity of the bladder, each being, of course, covered by a layer of bladder epithelium. At the same time the whole organ increases in size till it becomes about as large as the whole "green gland" of *Palæmon*.

A section through the adult end-sac is drawn in fig. 7, where the extent of the papilliform prolongations into which it is produced is rather below the average. The epithelium is flattened and irregular, composed of a pale, granular protoplasm. The cells do not exhibit vacuoles, and I have not observed the presence of concretions in any of my specimens.



The nuclei are small, rounded, and pale, with scattered, deeply staining chromatin granules. The communication between the cavity of the organ and that of the bladder is seen to lie at one side of the stalk, and to be quite direct, without the intervention of anything which can be held to represent the system of tubules of *Palæmon*, or even the simple U-shaped tubule of *Virbius*.

Between the wall of the end-sac and the investing portion of the bladder there is a certain quantity of connective tissue, which in places forms fairly conspicuous masses, the characters of which tissue will be gathered from the figure. Besides the connective tissue a system of blood-vessels ramifies between end-sac and bladder, consisting in part of larger vessels, in which it is easy to recognise an epithelial lining, and in part of smaller, apparently lacunar spaces. These vessels are supplied by one or two main trunks which pass along the neck of the end-sac. Two of these vessels, cut transversely before their entrance into the space referred to, are seen in fig. 7 lying outside the wall of the bladder.

The epithelium of the bladder itself is everywhere the same. The cells are columnar, and very regular in outline, showing no trace of vacuolation or of production into irregular processes. They exhibit an exceedingly well-marked longitudinal striation, and stain deeply both with hæmatoxylin and with borax carmine. The internal border of the bladder-cells seems always to be darker and more homogeneous than the rest, but there is no indication of the existence of a definite cuticle. The nuclei are rounded and granular, and stain fairly deeply.

The youngest individuals of *Crangon vulgaris* which were examined had already attained the external characters of the adult, although they were scarcely larger than the oldest "Mysis" larvæ.

These specimens correspond in age to the second stage in the development of *Pandalus*, as above described, and the condition of the excretory system is practically identical in the two species.

The bladder-wall in the young *C. vulgaris* (see Pl. XXI,

fig. 8) exhibits already the characteristic longitudinal striation, and as in the young *Pandalus* it surrounds half the end-sac. The communication between its cavity and that of the end-sac is direct, there being no trace of the renal tubule.

The end-sac is bounded by a layer of pale, finely granular cells, the protoplasm of which exhibits the well-known "ground-glass" appearance. The epithelium of that half of the sac which is enclosed by the bladder is more columnar, that of the unenclosed portion being flatter. The cells of both regions exhibit numerous vacuoles at their inner margins, but no concretions were observed.

Between the end-sac and the bladder is a well-developed, apparently lacunar blood-space, and outside the end-sac is a layer of connective tissue.

The excretory system of an adult shrimp resembles that of *Pandalus* in the direct communication between end-sac and bladder, and in the formation of papillæ upon that surface of the end-sac which projects into the bladder. The whole sac is not, however, enveloped by the bladder so completely as it is in *Pandalus*.

It is evident from what has been said that the excretory system of the Decapoda is much more varied in its structure than has hitherto been supposed. The observations here recorded, together with those of M. Marchal already referred to, enable us to divide the modifications into groups as follows :

In the Schizopods (*Mysis*) the whole excretory system appears, according to Grobben,<sup>1</sup> to consist of a single coiled tubule, opening by one extremity to the exterior, and by the other to an irregular end-sac, whose walls are composed of an irregular epithelium, and are not apparently very highly specialised. The single renal tubule may dilate into a small bladder near its external opening, but there is no indication of the extension into the thorax of a nephro-peritoneal sac.

In all the Decapods proper the end-sac has become more highly specialised, possessing a lining epithelium of definite characters, a well-defined system of blood-vessels, and so on ;

<sup>1</sup> ' Arb. Zool. Inst. Wien,' iii, 1881.

but the characters of the remaining portions of the excretory system vary greatly.

In the *Thalassinidæ* (*Axiu*s and *Gebia*) the number of the tubules, and the complexity of their arrangement, have increased, so that several tubules lead from the ureter to the end-sac; but there is no vesicular dilatation of any part of the system.

In the *Astacidæ* (*Astacus*, *Homarus*, and *Nephrops*) several tubules run from the ureter to the end-sac; but by the dilatation of one of these, which receives the openings of the others, a bladder is constituted; and this bladder intervenes between the plexus of tubules, forming the mass of the "green gland," and the ureter. In this group the bladder has no considerable extension into the thorax.

In the *Loricata* (*Palinurus*) the bladder has the same relations as those described in the *Astacidæ*; but the number and complexity of the tubules forming the green gland is very largely increased.

In the *Carididæ* the structure of the excretory system varies greatly, the four modifications described in the previous section of this paper all occurring within the limits of the group.

In the "*Anomura*" there is a well-marked nephro-peritoneal system, which in *Pagurus* forms a median dorsal sac, situated far back in the abdomen. The end-sac is frequently produced into papillæ, which are surrounded, not by a modified portion of the bladder, as in *Pandalus* and *Crangon*, but by a layer of renal tubules.

In the *Brachyura* the nephro-peritoneal sac sends well-developed prolongations into the thorax; there are, at least frequently, two such prolongations on each side, one dorsal and one ventral. The end-sac is frequently produced into papillæ, which are covered by the epithelium of the short, wide renal tubule, the cavity of this tubule being frequently broken up by a system of trabeculæ.<sup>1</sup>

<sup>1</sup> In this sketch of the various Decapods, the account of the *Thalassinidæ* and of the *Brachyura* is taken directly from Marchal's paper.

It appears, from the foregoing statements, that the nephro-peritoneal sacs of the Decapoda should be regarded rather as enlarged portions of a tubular system, such as that found in *Mysis* and in the *Thalassinidæ*, than as persistent remnants of a "cœlomic" body-cavity, into which tubular nephridia open.

The presence of coxal glands in *Nebalia*,<sup>1</sup> and of tubular nephridia in the zœca of *Eriphya*,<sup>2</sup> gives much interest to the search for an embryonic cœlom in these animals, which may be expected to behave like the cœlom of *Peripatus*.

## EXPLANATION OF PLATES XXI & XXII,

Illustrating Professor W. F. R. Weldon's paper on "The Renal Organs of Certain Decapod Crustacea."

### *List of Reference Letters.*

*Bl.* Bladder. *Br.* Brain. *B. v.* Blood-vessel (or lacuna). *Comm.* Circum-œsophageal nerve-commissure. *E. s.* End-sac. *Lbr.* Labrum. *N. p.* Nephro-peritoneal sac. *Æs.* Œsophagus. *St.* Stomach. *Tu.* Tubular portion of "green gland." *U.* Ureter.

FIG. 1.—Transverse section through the head of a *Palæmon serratus*, showing the connection between the dorsal nephro-peritoneal sac and the bladder.

FIG. 2.—Transverse section through the same specimen, rather behind Fig. 1, showing the dilated median ventral portion of the nephro-peritoneal system lying in the upper lip, and the dorsal portion lying above the stomach.

FIG. 3.—Transverse section through the head of *Pandalus brevirostris*, just in front of the œsophagus, showing the ventral mesentery formed by the two nephro-peritoneal sacs.

FIG. 4.—Horizontal section through the bladder and end-sac of an adult *Virbius*. The dotted lines indicate the course of the single renal tubule.

<sup>1</sup> Claus, 'Arb. Zool. Inst. Wien,' Bd. viii, Heft 1, 1888.

<sup>2</sup> Ebedinski, 'Biol. Centralbl.,' x, p. 178.



FIG. 5.—Horizontal section through the bladder and “green gland” of *Pandalus annulicornis* in the Mysis stage.

FIG. 6.—Transverse section through the kidney of *Pandalus annulicornis*, just after the final metamorphosis.

FIG. 7.—Section through the end-sac and associated wall of the bladder in an adult *Pandalus*. The letters *Bl.* lie in the cavity of the bladder.

FIG. 8.—Horizontal section through the kidney of a young *Crangon vulgaris*, just after the final metamorphosis.

FIG. 9.—Diagram of the excretory system of *Palæmon*.

FIG. 10.—Diagram of the excretory system of *Pandalus*.



The Nephridium of Lumbricus and its Blood-supply; with Remarks on the Nephridia in other Chætopoda.

By

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With Plates XXIII—XXV.

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THE nephridium of the common earthworm has recently been the subject of a contribution by Dr. Goehlich (20), in which he remarks on various statements made by Gegenbaur in his classical paper on the subject, published in 1853 (19), in which he corrected the then prevailing view that the segmentally arranged tubes were respiratory, and suggested their excretory function.

As several of Goehlich's statements are at variance with those of Gegenbaur I was led to look into the details of the organ, and I then found that on those points the more recent writer was in error, and that Gegenbaur's description is much more nearly correct than Goehlich's. In fact, I have not very much to add to the description of the earlier writer beyond matters of histological detail, and more especially the blood-supply, which Professor Lankester suggested that I should work out.

The work was carried out in the Zoological Laboratory of University College during the past eighteen months.

## CONTENTS OF PAPER.

1. Nomenclature of the parts of the nephridium, and the course of the various regions.
2. Histology of the various regions, and suggestions as to their function.
3. Comparison with the nephridium in other genera.
4. The nephrostome of *Perichæta malamaniensis*, n. sp., and other genera.
5. Circulation in the nephridium.
6. The nephridium of *Arenicola*.

# 1. NOMENCLATURE OF THE PARTS AND COURSE OF THE VARIOUS REGIONS.

The long winding tube which constitutes the excretory organ of *Lumbricus* is for the most part embedded in a coating of vesicular cells, which are in their turn surrounded by the pavement-cells of the cœlomic epithelium. The tube is, in its adult condition, as in its development, divisible into two portions: (I) a præseptal portion, consisting of the internal funnel or nephrostome and a short ciliated tube; and (II) a much more extensive post-septal portion. The latter is readily distinguishable into four regions: (1) the very long but narrow tube in continuity with the præseptal tube; (2) the short brownish ciliated middle tube; (3) the wide large tube; and (4) the muscular tube or duct, which opens to the exterior. The first three of these tubes (1, 2, 3) are twined about in rather a complicated but quite constant manner, and are bound together by the coat of vesicular cells in such a way as to form two great "loops," visible fairly distinctly with the naked eye when an opened worm is covered by spirit. Another "loop" is formed by the muscular duct (4). This last I will call the "first loop" (E, figs. 1, 2, 3); the "second loop" (F) contains the greater part of the narrow tube (1), and of the large tube (3); finally, the "third loop" (G) consists of a part of the narrow tube, a part of the large tube, and the whole of the middle tube (2).

The course taken by these various regions, from the funnel to the external aperture, and the relations of the tubes to the



loops, will be readily seen by a glance at fig. 2, which will convey more information than any verbal description. I will, therefore, pass on to the histology of the various parts.

I will premise that, with the exception of the funnel itself, or rather a part of the funnel, together with probably the muscular duct, the canal of the nephridium is "intra-cellular," i. e. the nephridium consists of a large number of perforated or "drain-pipe" cells, placed end to end (see Pl. XXIV, figs. 30, 31, 32). The size of the cell and the relative proportion occupied by the lumen constitute the main morphological differences observable in the various regions; but the character of the protoplasm is of very great physiological importance, although we do not fully know the exact function of each region.

## 2. THE STRUCTURE OF THE VARIOUS REGIONS.

I. The Præseptal Portion.—The nephrostome or funnel is carried at the end of the short portion of the "narrow tube" which passes through the anterior septum bounding a given somite. The character of this portion of the tube will be described below. This præseptal portion of the tube is surrounded by a mass of vesicular cells, the outlines of which are readily seen in the living condition, and frequently in stained preparations. These cells resemble those coating the post-septal portion, and appear to be similar to those described and figured by Kukenthal round the vessels and nephridia of *Tubifex* (24), and not improbably they have the same meaning. The superficial cells are usually flattened, and form a cœlomic epithelium continuous with that covering the septum.

The funnel (Pl. XXIII, figs. 4—7, and 32) has recently been figured by Goehlich (20) in some detail, but he has altogether misunderstood the appearance represented. As is well known, the funnel consists of a number of very long, narrow, somewhat wedge-shaped cells—which I shall call "marginal cells"—arranged around a central point. The nucleus in each is placed near the outer end of the cell, which is rounded

FIG. 1.—A nephridium treated with nitrate of silver, to show the pavement cœlomic epithelium covering the vesicular tissue, and forming in parts a suspensory fenestrated membrane, which carries blood-vessels. *E*. First loop. *F F*. Second loop. *G G*. Third loop. *N*. Nerve-cord. *S*. Septum. *SN*. Subneural blood-vessel. *V*. Subintestinal blood-vessel. *a*. Somatic branch from subintestinal blood-vessel. *d*. Ventral part of commissural vessel. *h, h, h*. Suspensory membranes, carrying and covering the nephridial blood-vessels. *i*. The group of cœlomic epithelial cells around the præseptal region of the narrow tube.

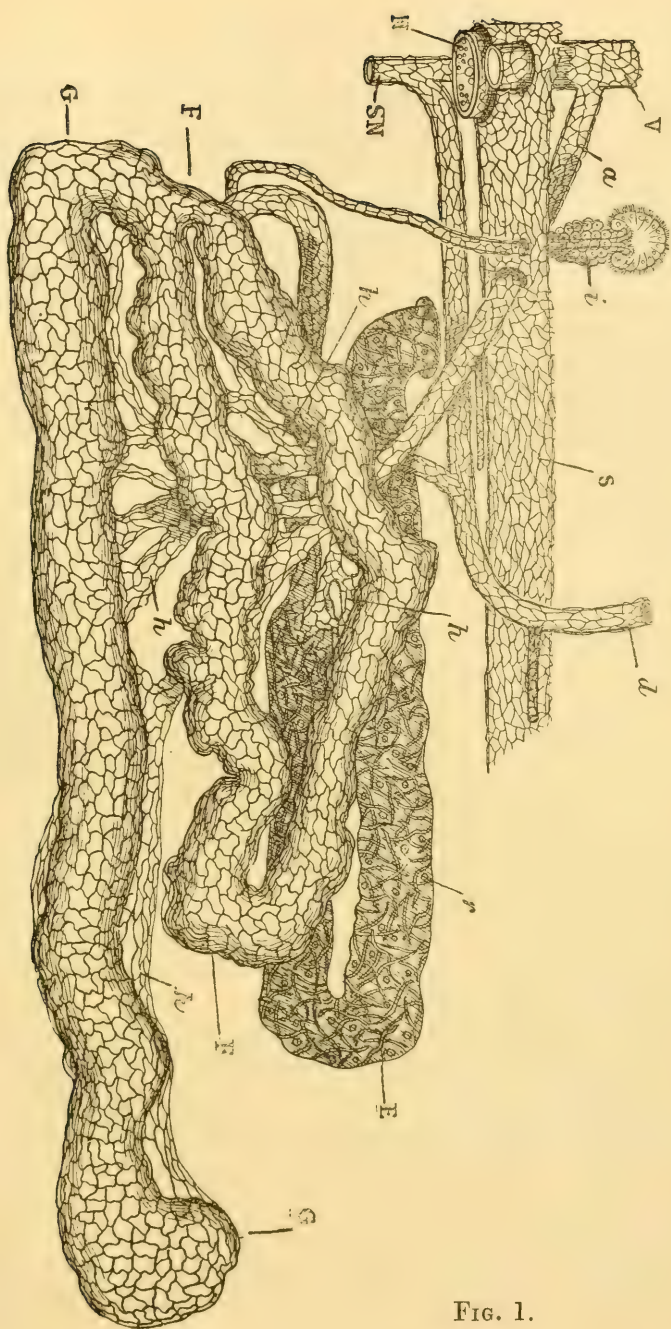


FIG. 1.

off peripherally, and ciliated all over one surface. Goehlich describes and figures one or two rows of specially long cilia radiating from the centre. These I have never seen, although I have had under observation hundreds of nephridial funnels in demonstrating to my students, and have examined carefully dozens for this very purpose. The appearance figured by Goehlich is due to a crumpling of the funnel—an action to which it is liable on being covered by a glass slip,—so that there will be caused radial folds, the cilia of which will then be seen sideways instead of from above, will therefore be more distinct, and will appear longer. There are normally no such longer cilia or such radiating lines.

Another phenomenon, which, indeed, renders the interpretation of the central portion of the funnel difficult, is the collection of a mass of cœlomic corpuscles in the funnel. This gives rise to the appearance represented in Goehlich's figure, and which was considered by him to form part of the wall of the funnel; but a careful examination, both of fresh specimens and stained preparations, has convinced me that these cells do not belong to the funnel, but are cells of the cœlomic fluid, in some cases dead or dying, which will probably be carried to the exterior, as Kukenthal has suggested. In the fresh state this group of cells or "débris" appears darker and less transparent than the cells of the funnel. Stained with borax carmine the nuclei have an appearance distinct from those of the nephridial cells, being brighter, more granular, and much smaller. In fact, many have the appearance of cells which have died, and have begun to disintegrate.

This mass of cells is shown in figs. 5 and 6. In one case (that represented by fig. 6), in which the funnel was examined twenty-four hours after the worm had been killed, not only were the cilia still active, but some of the cells forming the débris were still amœboid, one or two short pointed pseudopodia being present (fig. 6, *a*). Another case (fig. 5), where the cells of the débris are relatively few, shows the shape of the real mouth of the funnel, which is marked out by the aggregation of the cells into a crescentic mass. It was this



specimen that first led me to a better understanding of the real nature of the funnel, for no existing drawings or description give any clue to the real mode of communication between the *cœlom* and the nephridial tube.

I will now proceed to describe the true structure of the nephrostome.

If we follow the "narrow tube" forwards from the septum to the funnel, we see in optical section the finely granular wall on each side, with the nuclei of the component cells alternately on this side and on that. Arrived at the centre of the funnel, or thereabouts, the two walls suddenly diverge, each bending outwards, and then sharply backwards nearly parallel to its former course (fig. 4). The true "drain-pipe" cells cease at this point of divergence. The backwardly directed, or "centrifugal cells," as they may be termed, are merely grooved<sup>1</sup> (fig. 32), but otherwise resemble the drain-pipe cells in structure, being granular and ciliated. These grooved or "gutter" cells, in reality, lie in a different plane from the narrow tube, a higher plane when the funnel is viewed from in front; and they frequently hide the wall of the tube, as is the case in fig. 5.

After traversing a distance equal to about half the radius of the funnel the gutter-cells suddenly cease, and become continuous with cells having quite a different appearance (fig. 4); they are clearer and more distinctly marked off from one another. They are, in fact, similar to the marginal cells, with which they are continuous. The marginal cells are arranged in such a way as to form what at first sight looks like a circle, and has been figured as such; but what in reality is a horseshoe, the ends of which are bent inwards, the in-turned ends being very close to one another. The cells forming these ends may be called the "centripetal" marginal cells; and, as above mentioned, they meet the "centrifugal" gutter-cells (fig. 4).

The marginal cells are elongated, almost free from granules

<sup>1</sup> I. e. the lumen of the drain-pipe cell has enlarged; the wall on one side thins out till it parts, and the lumen is walled in only on one side.

FIG. 2.—The complete nephridium as seen (for the most part) in optical section, without any blood-vessels. It shows the character of the tubule in the different regions (note especially the ciliated tracts), the course of the tubule, and the arrangement of the constrictions, so as to form loops. *C*. The ampulla.

*E*. The muscular duct in the first "loop." *FF*. The second "loop." *GG*. The third "loop." *I*. The præseptal region, with the nephrostome. *a, b, c, d, e, f, g, h* point to different parts of the "narrow tube." *O*. The muscular duct, dipping into body-wall to open externally.

*a—b*. The first ciliated tract of the "narrow tube." *c—c'* (at the apex of the second "loop") is the second ciliated tract of the narrow tube. *d—e* is the narrow tube in the third "loop." *e—e'*. The third ciliated tract in the recurrent part of the narrow tube. *f* to *g*, to *h*. The remainder of the recurrent part of the narrow tube lying in second "loop."

*h—j*. The ciliated "middle" tube in the third "loop."

*k—l*. Part of the "wide" tube in the third "loop." *l—m*. The "wide" tube in the second "loop." *m—n*. The "wide" tube in the third "loop." *n* points to the junction of the wide tube with the muscular duct, where the tube is no longer seen in section, but in surface view. *r*. Nucleus of one of the cells covering muscular duct. *s*. A muscle-fibre, part of the network on the muscular duct. *t t*. Vesicular connective tissue, in which the tubule is embedded for the greater part of its course.

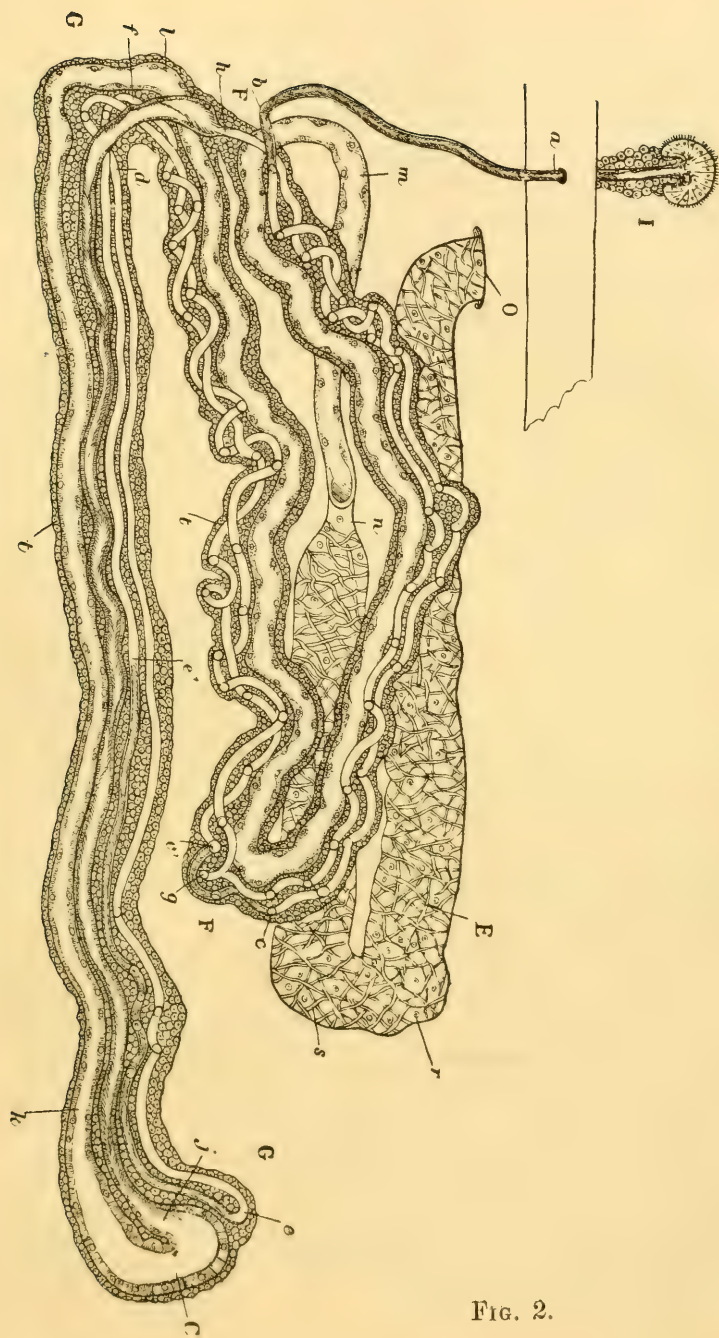


FIG. 2.

(hence are clear in the living state), with an oval nucleus; the cell boundaries are distinct, the outer free edge being slightly rounded, and the whole of that surface of the cell which is directed towards the funnel is ciliated. The centripetal marginals gradually decrease in size as the centre is approached, and become cubical, while the nucleus becomes round.

The inner ends of the marginal cells do not reach to the gutter-cells; and this space is usually figured, as by Goehlich (20), by d'Udekem (31, pl. iii, fig. 6), by Howes (in the 'Biological Atlas'), and others, as being occupied by numerous small rounded cells, considered as constituents of the funnel; these cells are, in truth, the "débris"<sup>1</sup> to which I have referred above, and have no structural relation to the funnel.

A very fortunate preparation shows the true structure of the back of the funnel (fig. 4); the space between the central ends of the marginal cells and the gutter-cells is occupied by one large crescent-shaped cell, with a particularly large oval nucleus containing a distinct nucleolus. This "central" cell is extremely difficult to see, for it is concealed by the débris; but having seen it in one funnel in which no débris was present, I have been able to detect it in other cases in which the débris was but slight; but generally it is completely concealed. In sections it can readily be recognised, as shown in fig. 7 (*Ce.*), which is the middle one of a series of sections through a funnel in a young worm.

The actual mouth of the funnel in communication between the cœlom and the nephridial tube is, therefore, placed between the inner edge of the central cell on the one side (the fine line *a* in fig. 4), and the outer edge of the grooved centrifugal cells of the other (the fine line *b*). The marginal cells and the general shape of the funnel are secondary, and are no doubt developed in order to influence by the ciliary current a greater extent of the cœlom. As to the probable steps in the

<sup>1</sup> Beddard has figured such a mass of cells in the funnel of *Urochæta* (3, pl. v, figs. 6 and 8; pl. xxiii, fig. 5), and has indicated it by "*pg.*;" he believes them to be cœlomic cells.



evolution of the funnel of *Lumbricus*, &c., I shall have a few words to say later on, in section 4.

The back of the funnel, as Vejdovsky has figured for *Allobophora rubida* (32, pl. xvi, figs. 15, 16), is covered by a flat cœlomic epithelium, the nuclei of which are readily distinguishable from those of the nephridial cells by their smaller size, and denser character of their chromatin network (fig. 7).

II. The Post-septal Portion.—(1) The “narrow tube,” which is the longest portion of the whole nephridium, possesses a thin transparent wall, the lumen being relatively to the diameter of the cell enormous (figs. 9, 10, 15). The protoplasm is finely granular; the nuclei, which can be seen readily in spirit or in moribund specimens at intervals on alternate sides along the tube, are fairly large, oval, and present a distinct nucleolus. This, in fact, is true of the nucleus of all the cells entering into the formation of the nephridium, as well as the tube itself, as in the funnel; the size and looser nature of the chromatin coil render them quite distinct from those of the surrounding connective-tissue cells.

For the greater part of its course the diameter of the lumen of the “narrow tube” is constant, but here and there certain portions have a very irregular lumen (figs. 8, 8a). In fact, in these cases, each cell, instead of being simply perforated by a straight tube, has a slightly branched lumen, a condition which is more marked in the nephridium of *Microchæta rappi*, as I have shown (8, pl. xvi *bis*, figs. 31, 33), as well as in other earthworms, and suggesting the complicated condition met with in *Clepsine*, *Hirudo*, &c.

Gegenbaur observed the fact that the cilia are not uniformly distributed along the whole course of the nephridial canal, but are limited to certain regions of the “narrow tube” and elsewhere. But in the exact limitations of these ciliated tracts he seems to have been in error.

Now Goehlich contradicts Gegenbaur, and maintains that the whole of the narrow tube is ciliated.<sup>1</sup> That this state-

<sup>1</sup> This is also implied by Hatchett Jackson in ‘Forms of Animal Life,’ 2nd edit., p. 204.

ment is absolutely wrong—at least for the nephridium of *Lumbricus herculeus*, Savigny (= *L. agricola*, Hoffmeister, = *L. terrestris*, Linnæus)—a careful examination of a living nephridium will readily show. How far the description of the excretory organ holds for all species of *Lumbricus* I am not yet in a position to say; but from examination of those of *Allolobophora*, sp., *Criodrilus*, and other genera, I can confidently state that the cilia are confined to certain limited tracts of the “narrow tube” (and to the whole of the “middle tube”).

There are three such ciliated tracts in the narrow tube: the first extends from the funnel to just beyond the point labelled “*b*” (in fig. 2)—it reaches, that is to say, just round the bend of the tube where the latter enters the second loop; this tract was quite correctly described by Gegenbaur. The second ciliated tract is at the apex of the middle loop (*F*), between the letters *c*, *c'* (fig. 2). I have observed that the extent of this tract varies to a very slight degree; but the cilia are always here, in the outer bend of the tube. The third tract of cilia lies in loop *G*; it commences (at *e*) where the narrow tube bends sharply upon itself, and extends about halfway along this loop, ceasing at *e'* (fig. 2).

Gegenbaur thought that the cilia recommenced at this point *e*, and extended right along to the end of the narrow tube.

It is comparatively easy to satisfy oneself as to the existence of these isolated tracts of cilia, either by observation on the living nephridium, or in series of sections; for if the whole tube were ciliated we should expect the cilia to be visible in every section of every tube if they were visible in any one section, but such is not the case; not only in *Lumbricus*, but in other genera of earthworms, I have sections in consecutive series which show cilia only in certain parts (fig. 15).<sup>1</sup>

Wherever these cilia occur they are arranged in a spiral

<sup>1</sup> Howes ('Biological Atlas'), in his figure of a portion of the third loop, shows correctly the structure of the different tubes, and represents one part of the “narrow tube” ciliated, and the parallel portion non-ciliated.

fashion, as Gegenbaur pointed out; not, however, along a single spiral line, but along two spiral lines. This can be seen in the fresh state, and in sections (figs. 9, 15); the cilia are directed outwards, i. e. from the funnel towards the muscular duct and external pore. D'Udekem (31) wrongly represents the cilia directed in the reverse way (pl. iv, fig. 9).

(2) The "middle tube" is quite short, occupying only one side of the loop *G*. The recurrent portion of the narrow tube, after following its previous course, arrives at the base of the second loop (*F*, fig. 2), and here enters a suddenly dilated region (at *h*), the commencement of the "middle tube," which extends only to the apex of the third loop (*G*).

The width of the lumen and of the wall of this region is a good deal greater than that of the narrow tube, but decreases slightly in size after its commencement. In the living state this middle tube has a brownish, semi-opaque appearance, and cilia can be seen actively moving within; under a high power small pale yellowish spherules can be seen in the protoplasm of the cells (fig. 11), and it is to these spherules that this middle tube owes its opacity. These spherules are of different sizes, the larger ones being nearer to the lumen. In the lumen itself, being carried onwards by the cilia, are numerous spherules, some smaller, others larger than those in the protoplasm, but otherwise similar to them (fig. 11). In transverse section, too (fig. 15), this region is readily distinguished from the other parts of the nephridium by the character of the wall, i. e. by the spherular products of the protoplasm.

Gegenbaur mentions these spherules as occurring in this and the following portion of the nephridium.

The whole of this tube is ciliated, and, as before, the cilia are arranged along two lines<sup>1</sup> (fig. 15).

(3) The "wide tube" commences at the apex of the loop *G*, and has the course shown in fig. 2.

<sup>1</sup> In Marshall and Hurst's 'Practical Zoology,' 2nd edit., the statement is made on p. 65 that this and the following regions of the tube have "proper cellular walls," implying that the lumen is intercellular. This is certainly not the case; the lumen is distinctly "intra-cellular."

At the apex of the loop the middle tube opens into a bladder-like enlargement (*C*) of the tube, called by Gegenbaur the "ampulla;" this is the commencement of the "wide tube." The ampulla is bent sharply upon itself, and gradually narrows in diameter till it reaches about twice that of the middle tube, which it retains throughout its course. There are no cilia in this part of the tube, notwithstanding Goehlich's statement that it is ciliated.

The wall of the ampulla is very different from that of the other regions. The cells are relatively very large, but are still "drain-pipe" in nature; their general arrangement and their shapes are shown in a figure taken from a living though moribund specimen (figs. 12, 30). The substance of the cell is divisible into two well-marked parts, a central and a peripheral portion (see figs. 13, 15), easily recognisable in a living nephridium. So marked is the distinction between the two parts, that in section it appears as if the perforated cells were surrounded by others, as Beddard (4) suggested might be the case in his description of *Allurus*; but such is not the case. A series of sections and examinations of living nephridia show that this appearance is really due to the modification of the protoplasm bordering the lumen.

The central portion of the protoplasm, which forms a thin lining to the lumen, is made up of numerous small granules arranged radially, giving the impression of a radial striation (figs. 13, 15). In preparations stained with borax carmine these granules take the dye much more strongly than the other parts of the nephridium, and give this ampulla a very characteristic appearance. The peripheral portion, which surrounds the central portion, forms a much greater bulk of the cell, and possesses much smaller granules, amongst which the nucleus is situated. The granules here also are arranged in rows, though less distinctly than in the central portion. A peculiar appearance, as of nuclei, is presented sometimes by this peripheral portion of the protoplasm: deeply stained, usually irregular, though sometimes regular, masses of different sizes are present (fig. 15). As a rule each mass, or sometimes a



group of small bodies, is surrounded by a clear space or vacuole. In the living nephridium the ampulla is seen to be filled with larger and smaller vesicles (fig. 13), which are probably the excretory products of the protoplasm of the tube; some, no doubt, pass into the ampulla from the preceding region—the “middle tube;” while others, and perhaps the larger globules, are, I believe, formed by the protoplasm of the cells of the ampulla, and the deeply staining masses in the peripheral portion of the protoplasm seen in sections are the above-mentioned globules or excretory products in situ, the substance being precipitated by the alcohol, stained.

Passing away from the ampulla, the central and peripheral portions of the protoplasm become gradually less distinct, till finally no such difference is to be observed; at the same time the wall of the lumen becomes relatively thinner. The granules in the protoplasm still retain a radial arrangement, though this, too, becomes less and less marked further from the ampulla.

In the greater part of the wide tube the wall is comparatively thin, but is not of uniform thinness; here and there it widens; in fact, each cell possesses a zone of wider protoplasm somewhere near its middle, in which the nucleus is situated; hence the margin of the wide tube is wavy (fig. 2). This was figured by Horst in his paper on the anatomy of *L. terrestris*.

(4) The muscular duct of the nephridium is considerably developed in *Lumbricus*; it is much wider than any of the preceding regions, and differs from these in the presence of abundant muscle-cells forming a trellis-work around the enclosed duct (*E*).

The lining epithelium of this region is very difficult to make out. It is usually stated that the lumen is here intercellular, and we should expect, if this be the case, to find a number of cells, with their nuclei fairly close together, arranged around the tube; but in *Lumbricus* we do not see this. After examining several preparations and series of sections in various directions, and treated in different ways, I still feel uncertain as to the true arrangement.

Frequently in a section, or even in several consecutive sections, no nucleus appears; then perhaps a section will show only one, or others show two, and very rarely three (fig. 16); these nuclei are quite similar to those in other parts of the nephridium—oval, with a nucleolus; and they appear to be embedded in a finely granular though very thin layer of protoplasm, showing a feebly reticular structure (fig. 16, *ep.*); in which, however, I can detect no cell boundaries.

Immediately outside this layer of protoplasm comes the muscular coat (*musc.*), which consists of cells arranged in various directions (fig. 16); some are longitudinal, others circular; still others are oblique, in relation to the long axis of the duct. These muscles appear also to be embedded in finely granular protoplasm (*cep.*), in which small round nuclei are scattered. It is difficult to make out a continuous layer of flattened cœlomic epithelial cells outside the muscular coat, either in section or in nephridia treated with nitrate of silver. From the appearance presented in many sections this granular protoplasm seems to represent the vesicular connective-tissue cells plus cœlomic epithelium of the other parts of the tube.

The muscular duct penetrates the body-wall, the muscles appearing to be continuous with those of the circular layer; a slight invagination of the epidermis meets the nephridial lumen, and puts the latter into communication with the exterior.

With a view to elucidating the character of the epithelium lining this part of the tube I have examined sections through other earthworms, and amongst these *Microchæta rappi*, in which the muscular duct is of enormous size (see my paper [8], pl. xvi, fig. 21). In this worm the lining of this region of the nephridium consists of very numerous vesicular cells, quite unlike what we have in *Lumbricus*; they are small, with distinct boundaries, and appear to be but loosely attached to the wall. This layer of cells can be traced through the body-wall up to the circular layer of muscles, where it meets the epidermic pit. I think, therefore, that we may conclude that in *Lumbricus*, too, the lumen of this region is intercellular,

although the cells are of very great size and relatively few in number, whereas in *Microchæta* they are small but very numerous: in fact, we have perhaps a stage in the transition between perforated cells and a more definite epithelium.

### 3. COMPARISON WITH OTHER GENERA.

Although we have, in numerous recent contributions, outline figures of the complete nephridium with the funnel of various *Oligochæta*, yet we have very few details as to the histological structure of the different parts of the tube.

Amongst the majority of the *Macrodrili* (in the "limicolous" *Oligochætes*) there appears to be no distinction of the nephridial tube into more than two regions: for example, amongst the *Enchytræidæ* (see Michaelsen's and Vejdovsky's works) we find (*a*) the post-septal portion consists of a more or less extensively convoluted "narrow tube" embedded in a mass of vesicular cells;<sup>1</sup> and (*b*) a præseptal portion, consisting of the simply constructed funnel and a short ciliated tube leading to the post-septal tube (see Pl. XXIV, fig. 27).

In *Tubifex*, however, where the coils of the nephridium are free, Vejdovsky (32) figures (*a*) a narrow region, (*b*) a middle or glandular region, and (*c*) a terminal or wide region, in addition to (*d*) the præseptal region. As in *Lumbricus*, the tube is surrounded by vesicular cells (see also Kukenthal, 24).

In the literature of the earthworms or *Macrodrili*, also, the detailed information as to the character of the tube of the nephridium is very scanty. Beddard (6) describes the different calibres of the tube in *Perichæta aspergillum* and *P. armata*, where three regions are evidently present, corresponding to the "narrow," "middle ciliated," and "wide" tubes; the last has striations in its wall. In *Ac. multiporus* a similar variation in calibre is noted (loc. cit., fig. 14), but only two regions are apparently distinguishable. Beddard

<sup>1</sup> Michaelsen, in his description of *Pachydrilus sphagnetorum* ('Arch. f. Mikr. Anat.,' xxxi, p. 491), states the whole of the tube is ciliated; and in *Enchytræus Möbii* and others, he figures this tube as slightly dilated just before the external aperture.



has also figured similar different regions in *Allurus* (4, pl. xxv, figs. 11, 12).

Spencer (30) describes and figures (pl. vi, fig. 26) for *Megascolides* the funnel and ciliated præseptal region of the large posterior nephridia, as well as the "wide" intra-cellular tube which passes into the body-wall as far as the circular muscles. At the junction of this layer with the epidermis a group of muscle-cells, arranged as a sphincter around the nephridial tube, is shown, and has a great resemblance to the condition found in *Criodrilus* (Pl. XXIV, fig. 17). Spencer also gives some details as to the opening of the small nephridia to the exterior, in which there is a short intercellular portion interposed between the epidermic pore and the intra-cellular tube.

Perrier some years ago (26, pl. xvi, figs. 38, 39) described with some detail the nephridium of *Urochæta*. He seems to imply that the whole tube is ciliated, with the exception of a very small muscular duct. More recently Beddard (3 and 6) has paid more attention to this genus. He indicates at least two regions (the "narrow" and "middle" tubes); he does not, however, represent the cilia, which I believe are present only in certain tracts, and not throughout.

*Pontodrilus* was also investigated by Perrier (27), who describes and figures (pl. xiv, figs. 10, 11, 13) the nephridia. The anterior nephridia (in somites 14, 15, 16, 17) differ slightly from the remainder, which have very abundant vesicular cells around the convolutions of the tube, much as in *Enchytræids*. The tube appears from his description to be similar throughout, for he speaks of it as a "sinuous glandular tube, ciliated internally throughout" its length. His figure of a section closely resembles that of the "middle" tube in *Lumbricus*.

In my first 'Studies on Earthworms' (8) I figured and described certain differences in the character of the tube in the nephridium of *Microchæta rappi*; and this genus I have re-examined, and can confirm my statements.

I believe the above are the only earthworms in which any attempt at histological detail has been made, and no author has



made any observations as to the different characters presented by different parts of the tube except Beddard. I have, therefore, examined sections through a number of earthworms of different genera, in order to ascertain how far there is any general agreement between their nephridia; and I find that such an agreement is present, with the exception that the muscular region exhibits a great variability, sometimes being entirely absent (as in *Criodrilus*), sometimes being enormously developed (as in *Microchæta*), and in other cases presenting intermediate conditions.

The nephridium of the undermentioned worms presents the same three regions that I have described for *Lumbricus*, viz. (1) a non-ciliated narrow tube; (2) a ciliated middle tube; and (3) a non-ciliated wide tube, which has frequently a wall of irregular thickness. In all cases the whole of these three regions is intra-cellular, and this may dip into the body-wall directly, or enter (4) an intercellular muscular duct as in *Lumbricus*. The funnel, however, presents a good deal of variety in structure, as I will describe below in section 4.

The worms I have examined<sup>1</sup> are *Criodrilus* (for a drawing of funnel see Vejdovsky, 32).

*Allurus* (see Beddard, 4, pl. xxv, figs. 11, 12, and Vejdovsky, 32).

*Microchæta* (see Benham, 8, pl. xvi, figs. 21 to 26; pl. xvi bis, figs. 31, 32, 33).

*Urobenus* (see Benham, 9, pl. viii, figs. 18, 19).

*Brachydrilus*.

*Rhinodrilus* (see Beddard, 1a, pp. 160, 161; and Horst, 21, fig. 8).

*Urochæta* (see Perrier, 26, pl. xvi, figs. 38, 39; Beddard, 3, pl. v, figs. 5 to 9; Beddard, 6, pl. xxiii).

*Diachæta* (see Benham, 9, pl. ix, figs. 27, 28).

*Perionyx*.

<sup>1</sup> It may be worth while to add references to the figures of complete nephridia, but which give no histological detail of other genera:

*Hormogaster*, Rosa, 28, fig. 8.

*Moniligaster*, Horst, 21, fig. 2.

*Eudrilus* (see Beddard, 2, pl. xxxiii, fig. 17; and Horst, 22, figs. 3, 4).

Some of these deserve further mention.

In *Criodrilus* the narrow tube is very much more extensive, and undergoes a greater amount of coiling than in *Lumbricus*, and herein rather agrees with the *Microdrili*. There is no muscular duct, the wide tube penetrating the body-wall as far as the circular muscles (Pl. XXIV, fig. 17). It is surrounded by a very loose connective tissue as it passes through the longitudinal muscles; and this connective tissue is modified just before the tube reaches the circular muscles. Here can be seen seven or eight, or perhaps a few more, nuclei belonging to certain cells, which are elongated transversely to the long axis of the tube (fig. 17, *y*); they resemble, in position and arrangement, the "sphincter muscle-cells" figured by Spencer around the tube of the large nephridia of *Megascolides*; but in *Criodrilus* they are certainly not muscle-cells.

At this point, when the tube has reached the circular muscles, it becomes difficult to be sure of the exact nature of the wall—i. e. whether it is formed by perforation of cells or by an epithelium; for the nephridial tube undergoes a slight flexure here, in order to reach the deep but narrow pit formed by invaginated epidermis, and constituting the nephridiopore.

A study of several series of consecutive sections through this region, however, gives me the impression that there is a very short intercellular tube between the wide tube and the epidermic pit: this then would correspond to the duct or muscular region of *Lumbricus* deprived of muscle-cells, the contraction of this part being, when necessary, brought about by the muscles of the body-wall. This anatomical difference between *Lumbricus* and *Criodrilus* seemed, when I first observed it, to agree with the different descriptions of the development of the nephridia given by various authors; some affirming the existence of an epiblastic invagination to meet the mesoblastic tube, others denying this invagination, e. g. Bergh in *Criodrilus* (12), where, indeed, we should not expect it to occur; but Bergh has quite recently (13) emphatically denied it also

in certain species of *Lumbricus* and *Allolobophora*, where he states that, as in *Criodrilus*, the whole nephridium is derived from mesoblastic cells which grow outwards, and ultimately pass between the epiblast-cells to reach the exterior.

A very interesting peculiarity in the "narrow tube" was figured by me in 1886 for *Microchæta rappi* (8), where I show the "smaller lumina" (*l'*, pl. xvi bis, figs. 31, 33), as I called this part of the tube, branching and anastomosing so as to form a network around the two other regions of the tube (*l. l'*).

No notice has been taken of this network in the recent discussions by Beddard and Spencer, as to the derivation of the nephridia of earthworms from a "plectonephric" condition, such as occurs in *Acanthodrilus*, *Megascolides*, and many others; and yet it seems to me not without interest.

Quite recently a similar condition of things has been mentioned by Rosa (29) in the nephridium of *Desmogaster*, one of the *Moniligastridæ*; Vejdovsky, too (32), figures the branching of the lumen in the case of *Chætogaster*.

Again, in *Brachydrilus*, where there are two pairs of nephridia in each segment, each having the same structure as that of *Lumbricus*, a similar network is formed by the "narrow tube" (see Pl. XXIV, fig. 26), and which is almost identical with that of *Perichæta*, &c.

Now what meaning is to be attached to this network in relation to the "plectonephric" condition? (1) Are the two networks homoplastic, i. e. has each been developed independently, or are they homogenetic? and if the latter, (2) is the "plectonephric" condition derived from the "meganephric" condition (of *Lumbricus*, *Urobenus*, *Eudrilus*, &c.) by the branching of the tubule; or (3) is it a remnant of a plectonephric condition still retained by the meganephric genera?

(1) The suggestion that the network in *Brachydrilus* and *Microchæta* (and no doubt this network will be found elsewhere) is homoplastic with that of *Acanthodrilus* might be held; but if the tubule can branch independently of any ancestral network, there is no reason to regard the plecto-



nephric condition of *Acanthodrilus* as related to that of *Perichæta* and other worms; and it would have no systematic meaning at all, but might have been developed in any genus, and at any period in its history. This assumption would, no doubt, explain many difficulties, such as the presence of a network in *Perichæta*, and of large nephridia in *Perionyx*, or the differences in different species of *Acanthodrilus*. In fact, the character of the nephridium would be no guide as to the affinities of worms.

(2) The assumption that the meganephric condition is archaic would, I think, plunge us into a sea of difficulties; for if a large nephridium is the more primitive, then we have to explain how it comes about that in some genera and species the paired nephridia are in relation to the inner couple of setæ, and that in others they are in relation to the outer couple; how is it that in some worms, e.g. *Perionyx saltans* and *Ac. novæ-zealandiæ*, the position of the nephridiopores alternates from segment to segment? and finally how can we explain the presence of two pairs of nephridia per segment in *Brachydrilus*? For if a large nephridium is ancestral, was there but a single pair, or two pairs, or four pairs? The last assumption would of course explain all the above queries.

If the plectonephric condition is derived from the branching of one pair of large nephridia per segment, then the double pair in *Brachydrilus* would have to be regarded as a secondary condensation of tubules after the appearance of a plectonephric condition; and the possibility would then arise that a large nephridium might be either primitive or secondary.

Ontogeny has not yet revealed to us any sufficient evidence on this point; the only plectonephric form studied is *Acanthodrilus*. According to Beddard ('Proc. Roy. Soc.,' 1890) there is at first a single pair of large nephridia in each segment, and these by branching give rise to the characteristic network. This looks like strong evidence in favour of Horst's view of an ancestral paired meganephric condition. But, as just stated, we should then have to meet the above difficulties



by supposing the branching and anastomosing of four pairs of large nephridia in order to give rise to the condition found in *Ac. multiporus*, where we have eight groups of tubules per segment; a further extension of the anastomosis leads to the more complete plectonephric condition of *Perichæta aspergillum*.

*Megascolides* would be explained by one of the four pairs remaining simple (in the posterior part of the body), the rest branching; still further, reduction to two pairs leads to *Brachydrilus*, and again to *Lumbricus*, *Urochæta*, &c.

But it seems to me that this is a strained hypothesis. For, after all, the occurrence in the ontogeny of *Ac. multiporus* of the paired nephridia may be merely cœnogenetic, and have no meaning of an ancestral nature; it would come in the same category as the formation of the heart in mammalia from a double rudiment.

The paired nephridia of *Acanthodrilus*<sup>1</sup> may, therefore, be merely provisional, like the embryonic nephridia described by Vejdovsky in *Rhynchelmis* and in *Lumbricus*, and those of embryo Pulmonates and other animals.

(3) It therefore remains for us to regard the branching tubes of *Brachydrilus* and other "meganephric" genera, as well as of the strictly "plectonephric genera," as homogenetic, and the plectonephric condition as the more archaic. This is the position taken up by Beddard and by Baldwin Spencer, although there is some difference as to details in the two theories.

Spencer has pointed out the parallel series of stages in *Oligochæta* and in *Hirudinea*; and it is interesting to compare the nephridium of *Hirudo* (see 14, p. 485) with its network of fine intra-cellular tubes around the main intra-cellular tube with that in *Brachydrilus*; and the less branched and non-anastomosing tubule in *Clepsine* (14, p. 483) with the condition met with in *Lumbricus* and other earthworms.

<sup>1</sup> If this be the case, then it would appear that the genital ducts in *Acanthodrilus* (and perhaps in other worms) arise, in part at least, by a modification of the embryonic provisional nephridia.

Again, one species of *Dinophilus* has been described as having a network of nephridial tubules; and another species, by Harmer, as having simple unbranched nephridia.

It appears possible that the meganephric condition may have arisen, as Spencer suggests (30, p. 43), either (*a*) by enlargement and elaboration of one tubule of the network, or (*b*) by an aggregation of a portion of the network.

Spencer does not state which alternative he believes to obtain in *Megascolides*, but probably the former, as he mentions no network in the large hinder nephridia, which he implies are simple unbranched tubules. He remarks that it would be impossible to map out the course of the tubules by means of sections: this might, however, be done by examination of the whole nephridium, as I have done for *Microchæta*; and I am inclined to regard the second alternative—aggregation of a portion of the network—as the probable mode of origin of the large nephridium (Pl. XXV, fig. 42). The tubule might become enlarged, lose some of its branches, and thus present a looser network; it would then become differentiated into “wide” and “narrow” regions, and ultimately lose all connecting tubules, so as to give rise to the complicatedly coiled tube of *Lumbricus*, &c.; while *Microchæta* and *Brachydrilus* would represent the intermediate stages with still some of the primitive network in part of the course.

Another intermediate condition appears to be presented by the “peptonephridium” of *Urochæta*, which retains some of the branches and three funnels, but has only one external aperture.

#### 4. THE FUNNEL OF *PERICHÆTA MALAMANIENSIS*, N. SP., AND SOME OTHER GENERA.

In my earliest contribution to lumbricological literature (8) I stated that a certain species of *Perichæta* from Malamani, amongst the Philippines, possessed numerous small nephridia in each segment with several funnels. I believe I was the first zoologist to mention this multiplicity of nephridial

funnels in *Oligochæta*, a character which, with the rapid extension of our knowledge during the last five years, has received considerable attention, owing to the careful researches of Beddard, and, more recently, of Spencer.

The species, of which I hope to give a more detailed account in a future paper, appears to be a new one, to which I give the above name. It agrees with *P. quadrigenaria*, Perrier, *P. elongata*, Perrier, and *P. exigua*, Fletcher, in possessing only one pair of spermathecæ; but it differs from each of these three species in various anatomical features.

As the funnel of the nephridium, which has the typical pleconephric character, differs from that figured by Beddard (6, pl. xxiii, fig. 10) for *P. aspergillum*, it appears worth while to give a figure of it (see Pl. XXIV, fig. 25, *a* and *b*). It consists of eight or nine marginal cells set in a circle around the terminal aperture of the tubule. All the cells are equal in size, and each is ciliated over the whole of the centrally directed face, the other face being covered by a few cœlomic epithelial cells. There is no "central cell," nor any centrifugal gutter cells. In *P. aspergillum*, however, the marginal cells are arranged in a horseshoe fashion, as in *Lumbricus* (see Beddard's drawing), but without the marked incurving of that genus, although a few smaller cells figured may represent either the completion of the circle or the "centripetal cells." However that may be, the funnel in this species, having a greater number of cells (some fifteen in number), which vary in size, is at a higher stage of development than that of *P. malamaniensis*.

I will add a few remarks and suggestions as to the funnel in various earthworms; for although there are but scanty materials for generalising, yet I think that, even with the few careful descriptions and figures, we can make out something of the evolution of the nephrostome in *Oligochæta*.

More or less careful figures of the funnel of *Perichæta*, *Megascolides*, *Urochæta*, *Microchæta*, *Urobenus*, *Criodrilus*, *Allolobophora*, and *Pontodrilus* occur in the literature (see above); but the majority of authors have



contented themselves with a brief mention of the fact of its presence, or given only a rough sketch of it. It appears to me that, just as the character of the nephridium itself in various genera is of interest and importance as representing to some extent probable stages in the phylogeny of the organ, so the nephrostome will indicate such steps.

Amongst the leeches, we know from Bourne's researches that *Clepsine* has a funnel composed of only two cells (14, fig. 52); other leeches show an increase in number of these cells; and *Hirudo*, with its more complicated nephridium, possesses a very large number of funnel-cells, each perforated or partially perforated by a branch of the nephridial tubule.

Amongst the Microdrili, such as *Tubifex* and *Enchytræus*, two is the normal number of cells composing the funnel. In *Rhynchelmis* there are eight such cells. In the plectonephric earthworms, such as *Acanthodrilus*, and the smaller tubules of *Megascolides* no funnels are present. In *Perichæta* from eight to fifteen marginal cells form the funnel; in the large tubules of *Megascolides* a larger number of cells are present, which from Spencer's drawing (30, pl. vi, fig. 26) appear to be arranged in a complete circle round the aperture, as in *P. malamaniensis*. In the higher meganephric condition we come across larger funnels, as a rule, with a great number of marginal cells, which form an incomplete circle, and differ in size according to their position.<sup>1</sup>

In my figure illustrating this anatomical point of *Urobenus* (9, pl. viii, fig. 19) I have drawn a number of cells curving inwards from the ends of the marginal cells, which are arranged in a horseshoe. These cells are no doubt the "centripetal marginals" and "centrifugal gutter-cells" of *Lumbricus*, although when I made the drawing I had a very imperfect idea as to their meaning. For *Urochæta*, Perrier (26, pl. xvi, fig. 42) gives a large drawing of the funnel, which presents a very wide aperture to the cœlom; the margin of

<sup>1</sup> An exception is to be noted in the case of *Microchæta rappi*, where the funnel seems peculiar, and may perhaps be degenerate.



the funnel is deeply incised at the front, and the cells forming this part are apparently quite continuous with the other marginal cells; but I think that it is very probable that the funnel of *Urochæta* is formed on the same plan as in *Lumbricus*. Perrier was unable to distinguish any nuclei in the wall of the widely dilated portion of the tube immediately below the margin of the funnel—between, that is, the nuclei of the marginal cells and those of the intra-cellular tube itself,—and suggests that the marginal cells are extremely elongated.

Beddard (3 and 6) regards this dilated region as being intra-cellular. In the former paper he gives figures of sections through the funnel of the ordinary nephridia, in which there is—contrary to Perrier's figure—no very marked dilatation of the tube; but the funnel appears to consist (fig. 7) of several rows of non-perforated cells (although such is, perhaps, not intended, and probably is not the case). Of the peptonephridium, Beddard (6) figures the funnel, and there he shows the perforated cells (? = "grooved cells") diverging from one another, so as to give rise to a very wide "intra-cellular" tube before the actual aperture is reached. The whole margin is formed by one row of marginal cells, and he makes no mention of the incurving of the margin represented by Perrier.

For *Pontodrilus*, Perrier (27, pl. xiv, fig. 12) figures only the side of a nephrostome covered by coelomic epithelium, and gives little detail in the text; from it I cannot be sure whether the perforated cells reach to the marginal cells simply as in *Perichæta*, or whether there is any widening of the tube, as in *Urochæta*.

In *Rhinodrilus* (*Thamnodrilus*), Beddard (1*a*, p. 161) gives a figure of a complete nephridium, and describes the funnel as an "elongated, folded membrane, composed of ciliated columnar cells." I have removed a nephridium from a specimen of the same species (*Rh. gulielmi*); and I can confirm this statement, but will add that the funnel is quite similar to that of *Lumbricus* (with marginal cells, centripetal and centrifugal cells) if we imagine it to be, not circular, but drawn

out on each side to a very considerable extent, so as to be two or three times as wide from side to side as from front to back.

Even with such scanty details as we have, I think it is possible to trace a few stages in the evolution of the complicated funnel of *Lumbricus*, *Rhinodrilus*, &c. Starting with the nephrostome of the *Enchytræidæ*, for example, we have a funnel formed merely by the terminal perforated cell without accessory marginal cells (see Pl. XXIV, figs. 27, 28, 29, *a*). By the subdivision of this cell, as in the ontogeny of *Stylaria*, the lip of the aperture is formed by two cells (as is the case also in *Clepsine* amongst the leeches); in *Tubifex* also two or a few more (a similar increase is observed in *Pontobdella*). In *Rhynchelmis*—one of the nearest of the waterworms to earthworms—eight marginal cells have become formed, as is the case, too, in *Per. malamaniensis* (Pl. XXIV, fig. 25); now a differentiation occurs amongst the cells, as in *P. aspergillum*. In *Urochæta*, in the peptonephrostome, not only have the marginal cells greatly increased in number, but the intra-cellular tube is dilated (see diagram, Pl. XXIV, fig. 25, *b*), so as to increase the effect of the ciliated margin. Suppose this dilatation were carried further, so that the sides diverge, the “perforated cells” would become “grooved cells,” and would lie between the intra-cellular tube and the marginal cells (diagram, fig. 25, *c*). I am not sure that any of the earthworms studied correspond to this stage. A little further differentiation, by a still further divergence and an outward curve of the “grooved cells,” leads on to the stage presented to us by *Rhinodrilus* and *Lumbricus* (cf. diagram, fig. 25, *d*, with Pl. XXIII, fig. 4). These large nephrostomes replace the numerous small ones of *Perichæta*, such being evidently more efficient for the removal of phagocytes than is the smaller funnel of the latter genus.

##### 5. THE VASCULAR SUPPLY OF THE NEPHRIDIUM.

The living nephridium of an earthworm is a remarkably beautiful object when viewed under a moderately high power; and this is due to the delicate and complex network of blood-

vessels which twine amongst themselves and between and around the tube of the nephridium (fig. 3).

Although the existence of this elaborate blood-supply has long been known (for instance, Morren describes it), and was indeed adopted by Claparède as one of the distinctive characters of earthworms as opposed to water-worms, yet no drawing or detailed description appears to have been published.

The remarkable dilatations of the blood-vessels here and there were observed by Williams long years ago; they received further attention from Lankester and from d'Udekem, who figured them (31, pl. iii, fig. 27), and a little later from Claparède (16). In *Pontodrilus*, Perrier (27, pl. xvii, fig. 35) observed them; and in *Diachæta*, I pictured them (9, pl. ix, figs. 35, 36).

It is to be noted that these dilatations are by no means constantly present in every earthworm. I have frequently examined dozens of nephridia from one specimen of *Lumbricus*, and seen no dilatations whatever (as fig. 22); whilst in other specimens examined at the same time, from the same locality, and apparently in the same sexual condition, I have seen these dilatations in abundance. But, again, not every nephridium in the same worm presents this character; in some they are rare, in other nephridia they are altogether absent.

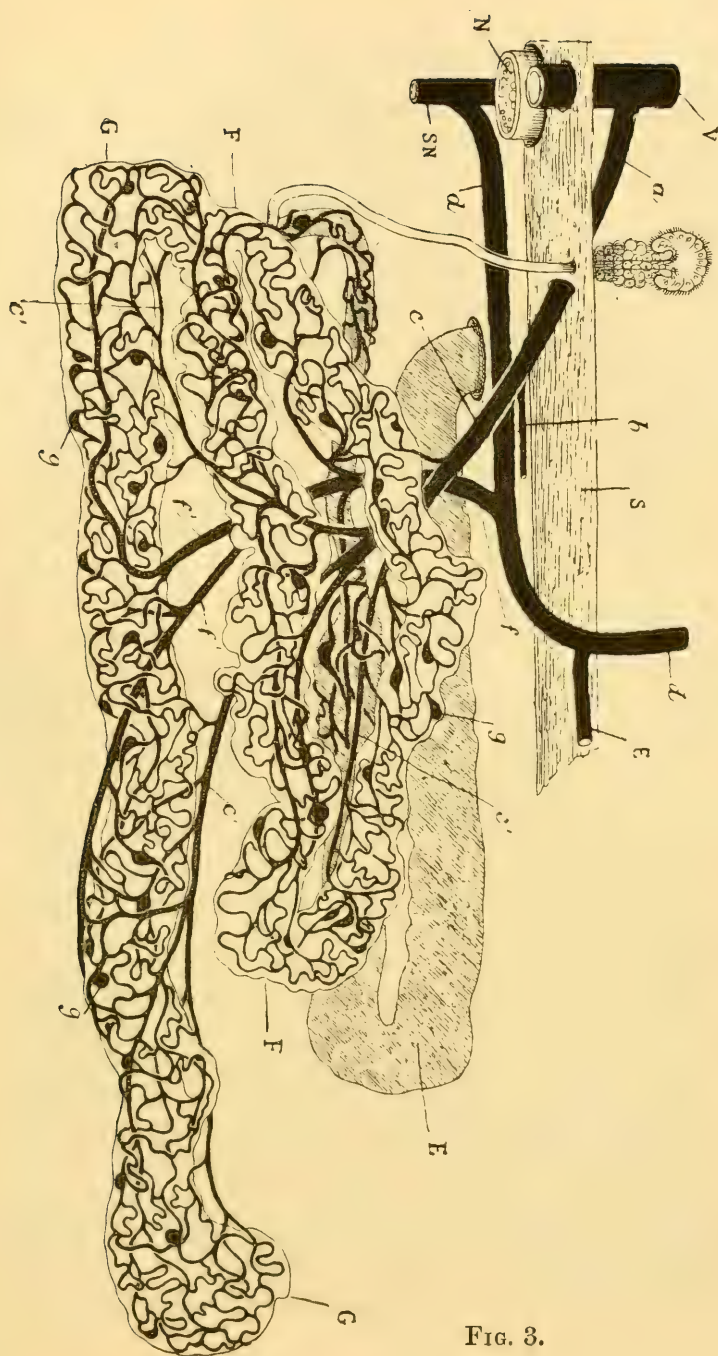
As is well known, they occur not only on the nephridia, but on the septa; and are, as Claparède figured, usually filled with corpuscles (as in Pl. XXIV, fig. 18).

The cause of their appearance and the use of them to the worm is a matter, at present, of speculation. Gegenbaur (19) noted them and their inconstancy, and suggested that they are present when the gonads are fully ripe; but they seem to be independent of this condition.

Another peculiar phenomenon is sometimes to be seen in the blood-vessels in the nephridium; the colour of the blood, instead of being pale yellow, as is normally the case, is more distinctly red—a pinkish red—comparable to the colour of

FIG. 3.—The vascular supply of the nephridium, the loops of which are represented in outline. *E*. The first loop. *FF*. The second loop. *GG*. The third loop. *N*. Nerve-cord. *S*. Septum. *SN*. Subneural blood-vessel. *V*. Subintestinal blood-vessel. *a*. Somatic vessel from subintestinal vessel. *b*. Its septal branch. *c*. Its nephridial (afferent) branch. *c' c'*. The further subdivision of this. *d*. Ventral portion of a commissural vessel, connecting the dorsal vessel with the subneural. *e*. Its septal branch. *f*. Its nephridial (efferent) branch. *f' f'*. Its further subdivisions to third loop. *g g'*. Dilatations in the capillary vessels.





venous as opposed to arterial blood ; and in fact, as Professor Lankester suggested to me, this is probably due to the reduced condition of the hæmoglobin in the blood.

The vessels supplying the nephridium, as shown in the diagram (fig. 3), are connected with the subintestinal vessel (*V*) on the one hand, and with the dorsal vessel and subneural vessel (*SN*) through the commissural vessel (*d*) on the other.

It is a difficult matter to decide what course is taken by the blood outside the large median trunks, where it flows forwards in the dorsally, and backwards in the ventrally situated vessels ; it also passes downwards from the dorsal to the subintestinal vessel in the lateral hearts ; but what is its course in the commissural vessels in the segments behind the gizzard ?

A couple of years ago, when studying the arrangement of vessels in *Lumbricus*, I succeeded after considerable trouble in injecting the dorsal vessel in two specimens ; the blue injection passed forward readily, but only in one or two segments did it pass into the commissural vessels. I have a note that "internal intestinal plexus and typhlosolar sinus received injection," but that only "in a few cases did injection appear to pass on to the wall of intestine." By observation of the narcotised worm it can readily be seen that blood flows into the dorsal vessel from the commissural vessels, and this is confirmed by the arrangement of the valves at the entrance.

The course of the blood appears to be different in *Microchæta*, *Urochæta*, and *Megascolex*.<sup>1</sup>

I believe from the above experiments and observations (which I intend to pursue further) that the blood usually passes from the subneural (*SN*) into the dorsal vessel by way of the commissural vessels (*d*) ; the latter receives, amongst other branches, one from the body-wall (*e*), another from the nephridium (*f*) ; the blood is therefore aërated in the body-wall and denitrogenised in the nephridium before it reaches the dorsal vessel.

The blood, in passing backwards along the subintestinal trunk (*V*), passes outwards through the lateral (*a*) branches to the nephridium (*c*), and to the body-wall (*b*). The former

<sup>1</sup> A. G. Bourne, 'Quart. Journ. Mier. Sci.,' 1891, February.

divides up into branches to the three loops of the nephridium, and these branches subdivide; the ultimate capillaries wind in and out around the individual tubes, or around the whole bundle of tubes in a loop, as seen in figs. 19—24.

The blood-vessels to and from the nephridium pass along fenestrated membranes as shown in fig. 3, at *h*, which thus serve also to suspend the nephridium. In some cases I have seen a number of small twigs given off from a vessel in the septum, passing to the nephridial vascular network, in addition to the main supply (fig. 23).

An examination of the various figures will, I think, suffice to explain the relations and variations of the elaborate and delicate vascular network, and will render further remarks unnecessary.

## 6. THE NEPHRIDIUM OF ARENICOLA.

While I was working in the laboratory of the Marine Biological Association at Plymouth, during a short time in last summer, I took the opportunity of examining the nephridia of some of the readily obtainable Polychæta, amongst them *Nereis*, *Aphrodite*, and *Arenicola*, and I here introduce a figure of the nephridium of the last worm for comparison with that of *Lumbricus*.

The figure (Pl. XXV, fig. 33) is drawn under the low power, and exhibits, amongst other points, the elaborate vascular network, whilst the sections demonstrate the intercellular character of the wide lumen of the organ.

The nephridium of *Arenicola* has recently been described by two authors, namely, Cosmovici in 1879 (17), and Cunningham in 1887 (18); and I have, indeed, nothing to add to their descriptions. Cosmovici figures the nephridium in situ (pl. xx, fig. 10), and gives various drawings illustrative of the minute anatomy; though, as Cunningham has pointed out, his description of the lining epithelium is incorrect, inasmuch as he considered it to be stratified, which is not the case.



Both the above-named authors figure and describe the network of blood-vessels on the funnel and the body of the nephridium, but neither mentions the blind dilated terminations and irregularities in the capillary vessels (Pl. XXV, fig. 34), recalling those in *Lumbricus*; Cosmovici, however, does mention these as occurring elsewhere in *Arenicola*.

Notwithstanding the general accuracy of the descriptions given by these authors, I have thought it worth while to introduce another figure of the whole organ, which to my mind represents the form and relations of the parts more clearly than either of those already published.

The nephridium of *A. piscatorium* (Pl. XXV, fig. 33) consists of (*a*) a large, wide, tapering tube or "body," lined by a single layer of ciliated vesicular cells (fig. 40); (*b*) of a very wide funnel attached to the broad end of the "body" by a narrow "neck;" (*c*) of a dilated, more muscular region, opening to the exterior and separated from the narrow part of the body by a well-marked constriction.

A very noticeable contrast to the nephridium of *Oligochæta* is presented by that of *Arenicola* and other *Polychæta*, e.g. *Polynoë* (A. G. Bourne, 15, pl. xxvi, figs. 22, 23, 24), in that the lumen is intercellular throughout; and this point deserves mention because Baldwin Spencer (30, p. 44) has argued against the theory that the genital ducts of *Oligochæta* are modified nephridia, because, whilst the lumen of the latter is mainly intra-cellular, that of the former is wholly intercellular; yet, I imagine, he would not deny that the nephridia in the two groups are homogeneous; and they appear to serve as genital ducts in some *Polychæta*.

The wide "body" contains no convoluted tube, as is the case in *Nereis* and other genera, but consists of one very wide, simple tube, as is readily to be seen in sections through the organ (Pl. XXV, figs. 35, 36, 37). The epithelium is one cell deep, is ciliated throughout (figs. 40, 41), and for the most part contains brown concretions (fig. 41), giving the whole organ a yellowish colour. In the lumen of the gland, in section, is a granular mass, frequently having the appear-



ance of a network ; this is probably the coagulated excretory product.

The structure of the "bladder," or posterior terminal dilated portion, is similar to that of the "body," but the concretions are fewer, and as the external aperture is approached are almost absent ; the walls of this region are, too, provided with muscles.

The anteriorly directed funnel is very large, and consists of neck and lips. The two lips are dorsally and ventrally situated with regard to one another. The dorsal lip carries a large number of delicate processes, sometimes simple, sometimes bifid or multifid, and even arborescent. The ventral lip, on the other hand, is entire ; the slight notches, &c., in the drawing represent the folds resulting from compression.

The cells forming the edges of the two lips differ ; the dorsal lip is lined by ciliated vesicular cells (fig. 40), similar to the general lining epithelium of the body, but with numerous cilia, and without concretions.

The cells lining the ventral lip, on the other hand, are short, columnar, pigmented, and ciliated (fig. 39), whilst at the extreme edge the cells become more cubical, large, and are deprived of pigment granules (fig. 38).

I hope, in the near future, to examine chemically the products of excretion in *Arenicola* and other Polychætes.

I have (fig. 36) drawn one of many sections showing ova in the neck of the nephridium ; however, I found none in the body or in the "bladder," and it may possibly be that the funnel merely retains the ova for a period, and does not serve as a genital duct. I think it probable that they really pass to the exterior by the nephridium.

Cosmovici and Cunningham have given some details of the vessels supplying the nephridium. The nephridial vessel is a branch of the branchial vessel which is given off by the sub-intestinal vessel, much in the same way as in *Lumbricus* (Cosmovici, loc. cit., figs. 2—10).

The network on the nephridium lies between the excretory epithelium within and the flat cœlomic epithelium outside.

The funnel itself is particularly well supplied with blood, more especially the dorsal lip, which is traversed by one of the main branches of the nephridial vessel, as shown in the figure; the other branch (*a'*) passes along the gonad, which is not figured, and enters (at *b*) the general network in the nephridium.

A portion of this network is exhibited at fig. 34, which is an enlarged view of the vessels on the neck of the funnel.

This network is present in many Polychæta, as *Cosmovici* and others have pictured.

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## EXPLANATION OF PLATES XXIII—XXV,

Illustrating Dr. W. Blaxland Benham's paper on "The Nephridium of *Lumbricus* and its Blood-supply; with Remarks on the Nephridia in other *Chætopoda*."

(Where not otherwise stated, the drawings refer to *Lumbricus herculeus*, Sav.)

FIG. 4.—The nephrostome or internal funnel, seen from in front and represented as slightly flattened, so as to show distinctly the various parts. Cilia are omitted for the sake of clearness. The figure is fully named. The central cell is left white. The vesicular connective-tissue cells (*ves.*) around the præseptal portion of the tube are represented only by their nuclei. *n. cæl. ep.* is the flattened cælotomic epithelium. The two fine concentric lines near the centre of the funnel represent the boundaries of the actual aperture, which places the tube in communication with the cælotom, and which is further shown by the arrow. The line *a* is the inner limit of the large "central cell," its outer limit being formed by the inner ends of the "marginal cells." The line *b* is the thin outer free edge of the series of grooved centrifugal cells, which are essentially drain-pipe cells, open along one side, or gutter-cells. These two lines require not only a high power (Zeiss, 3, F), but a particularly good clear preparation for their detection.

FIG. 5.—A nephrostome drawn during life, to show the collection of leucocytes or "débris" blocking the actual aperture of the funnel. These have misled previous observers (see text). The cilia on the marginal cells are to some extent diagrammatically represented, as only a few rows are drawn; in reality they cover the whole of the inner surface of the cells. The "gutter-cells" are hidden by the superposed centripetal marginals.

FIG. 6.—A funnel in profile, from a living specimen, showing leucocytes or "débris."

FIG. 6*a*.—A few of these leucocytes, showing pseudopodia.

FIG. 7.—A longitudinal section through a funnel, from a series of sections through a young worm [? sp.] (which were cut by my friend Mr. E. Goodrich). The figure is fully explained.



FIG. 8.—A portion of the apex of the second loop, from a living nephridium (Zeiss, 4, B), in order to show the second ciliated tract, *c* to *c'* (see also Fig. 1), in the outer narrow tube; together with the adjacent non-ciliated portions. *ves.* Vesicular connective-tissue cells.

FIG. 8*a*.—A portion of another nephridium, to show the irregular character of the lumen (? remnants of branchings) of the narrow tube.

FIG. 9.—A portion of Fig. 8 under a higher power (Zeiss, 4, E), showing the character of the wall.

FIG. 10.—A portion of Fig. 9—a living nephridium—still further enlarged, to show the termination (*c*) of the ciliated tract at the apex of the second loop. This tube at *c* is seen in optical cross-section owing to its sudden bend.

FIG. 11.—A small portion of the "middle tube" in optical section, during life (Zeiss, 4, E). It shows the character of the protoplasm, the limits of the cells, the excretory products in the protoplasm and in the lumen, and the double row of cilia. The arrow points towards the ampulla (see Fig. 1).

FIG. 12.—The surface of a portion of the ampulla (drawn from a living nephridium under Zeiss, B). It shows the shape and boundaries of the constituent perforated cells.

FIG. 13.—An optical section of a small portion of the ampulla (drawn from a living nephridium with Zeiss, E). The differentiation of the protoplasm of the cells into "central" and "peripheral" regions and the radial arrangement of the granules are shown. The circles in the lumen represent the spherical excretory products.

FIG. 14.—An optical section of the "wide tube," drawn from a living nephridium (under Zeiss, E).

FIG. 15.—A transverse section across the third loop, near its apex, at about the level of *j* in Fig. 1 (from a series stained in borax carmine; drawn with Zeiss, 4, E). It is fully explained in the Plate. The middle tube shows the characteristic spherular character of the protoplasm of its wall, and the two rows of cilia; the granules in the lumen are the remains of excretory products. *b. v.* Blood-vessels. *n.* Nuclei of their endothelium. *c. t.* Vesicular connective-tissue cells, the limits of the cells not being visible in the sections. *nc.* Their nuclei. *c. ep.* Nuclei of cœlomic epithelium. *x.* A curious fibrous appearance sometimes visible in sections.

FIG. 16.—A transverse section through the muscular duct (from the same series as Fig. 15, under Zeiss, 3, F). *ep.* The lining of the duct. *mus. c.* Muscle-cell. *n. mus.* Nucleus of muscle-cell. *c. ep.* represents cœlomic epithelium.

FIG. 17.—A portion of a transverse section through the body-wall of *Criodrilus lacuum*, in the clitellar region, in order to show the absence of a muscular duct in this genus, and the consequent perforation of the longitudinal muscles by the "wide tube." *y* is a group of connective-tissue cells,

which hide the small intercellular part of the nephridium intervening between the "wide tube" and the epidermic pit forming the nephridiopore. *cl.* Clitellar cells. *ep.* Epidermis. *con. tis.* Connective tissue. *circ.* Circular muscles. *lg. mus.* Longitudinal muscles. *neph. pore.* Epidermal pit, constituting the nephridial pore (which is deeper in the neighbouring sections).

FIG. 18.—A portion of the nephridial loop of *Lumbricus* in longitudinal section, passing through some of the dilatations of the blood-vessels, in which are groups of blood-corpuscles.

FIG. 19.—A transverse section through a part of the nephridium on one half of the second loop, showing the blood-vessels ramifying between the different parts of the tube.

FIG. 20.—A similar section through the tip of the third loop, to show blood-vessels ramifying between the different parts of the tube.

FIG. 21.—The apex of the third loop, to show blood-vessels passing among and around the various parts of the tube. It is slightly diagrammatic. Those portions of the blood-vessels which pass behind the tubes are represented only in outline.

FIG. 22.—A portion of a nephridium, drawn from a living specimen under a high power, to show the complexity of the coils and branchings of the capillaries; there were no dilatations in this specimen. The outlines, merely, of the nephridial tube are indicated.

FIG. 23.—Portion of a loop of a nephridium of another worm, in which the dilatations of the blood-vessels were numerous (drawn under a low power from a living specimen). Many of the finer vessels are not shown, nor are the outlines of the nephridial tube. A portion of the septum is shown, with small blood-vessels passing across from it to the nephridial network.

FIG. 24.—A portion of the same network greatly enlarged, to exhibit the varying size and shape of the dilatations.

FIG. 25.—The nephrostome of *Perichæta malamaniensis*, n. sp. *a.* In longitudinal section. *b.* From in front. These drawings are taken from the same transverse section through the worm, and the two funnels are quite close together, some five or six occurring in the whole section. The drawing is introduced to show the simple character of the nephrostome when compared with that of *Lumbricus*. *ma.* Marginal cells. *ap.* Communication between nephridial lumen and the cœlom.

FIG. 26.—A portion of a section through a nephridium of *Brachydrilus*, to exhibit the branching of the narrow tube which wraps round the wide tube. The blood-vessels are also shown in black or in grey, according to the relative depth in the section.

FIG. 27.—A nephridium of an *Enchytræid*, to demonstrate the simple character of the nephrostome (*st.*), the uniform diameter, &c., of the tube (*t.*), which is embedded in one mass of vesicular cells (*c. t.*). The external aperture is shown at *p.*

FIG. 28.—The nephrostome of an Enchytræid (modified from Michaelsen) in surface view, to show the nephrostome formed by the terminal perforated cell.

FIG. 29.—A series of nephridial funnels in optical section, diagrammatically represented to exhibit the possible evolution of that of *Lumbricus* and others (*d*) from that of an Enchytræid (*a*). The intermediate form (*b*) is met with in *Urochæta*; that represented by *c* is, as far as I know, hypothetical. Between *a* and *b* that of *Per. malamaniensis* may be inserted.

FIGS. 30—32.—Diagrammatic views of constituent cells of a nephridium.

Fig. 30. A perforated (drain-pipe) cell, as met with in the ampulla.

Fig. 31. A drain-pipe cell from the middle tube.

Fig. 31*a*. The same in longitudinal section.

Fig. 32. A gutter-cell from the nephrostome of *Lumbricus*.

#### *Arenicola piscatorum*.

FIG. 33.—A complete nephridium. Surface view, under a low power. The specimen was removed from the body, stained in borax carmine, and mounted in Canada balsam. The natural size is shown by the line (*n. s.*) underneath. The nephridium is seen from its ventral side, and the gonad, which in life is closely connected with it, has not been drawn. The network of blood-vessels, in black, is drawn as far as I could make it out; no doubt it is more complicated and extensive than is shown. *a* is a branch of the branchial vessel, passing to the nephridium. *a'* is a branch to the gonad, passing behind the nephridium. *b* is the other end of this (cut) after passing through gonad to join the network in the nephridium. *mes.* is a small piece of mesentery, which suspends the funnel to the ventro-lateral muscle.

FIG. 34.—A portion of the network in the "neck" of the nephridium, drawn under a higher power, and showing the cæcal dilated terminations (*di.*). The cœlomic epithelium (*cœl. ep.*) is drawn in part of the figure.

FIG. 35.—A longitudinal section through the body of the nephridium, with the ovary in situ. The blood-vessel marked *b* is intermediate between *a'* and *b* in Fig. 33.

FIG. 36.—A section through the neck of the funnel, with a couple of ova within.

FIG. 37.—A section along the funnel and neck, with neighbouring parts of the body-wall and ventro-lateral muscle (*mus.*). The letter *p* points to the relative position of the nephridiopore, which occurs several sections further back. *o.* is an ovum.

FIG. 38.—A portion of the epithelium on the edge of the ventral lip of the funnel (from Fig. 36, under high power). *n.* Nucleus.

FIG. 39.—A portion of the epithelium lining the ventral lip (under high power). *n.* Nucleus.

FIG. 40.—Similar section through dorsal lip.

FIG. 41.—A portion of the lining epithelium of the body of the nephridium

(under high power). *b. v.* Blood-vessels. *cœl. ep.* Nucleus of flat cœlomic epithelium. *conc.* Coloured concretions in the vesicular renal cells (*epith.*). *n.* Nucleus of these cells. *exc. pr.* A granular coagulum, probably excretory product in nephridium.

FIG. 42.—Diagram to illustrate the suggested evolution of a single nephridial tube from an aggregation of a group of plectonephric tubules. *a.* A portion of primitive network of a segment. *b.* One of the many funnels occurring in each segment (as in *Perichæta*). *c.* One of the numerous ducts passing to exterior. *b'.* One of the funnels which persists and has become increased in size by addition of marginal cells. *c'.* The ultimate single duct. *e.* The tube (darkly shaded) of meganephric condition. *f.* A few of the anastomoses (dotted) remaining, as in *Brachydrilus*, &c.



Notes on the Naidiform Oligochæta; containing a Description of New Species of the Genera *Pristina* and *Pterostylarides*, and Remarks upon Cephalization and Gemmation as Generic and Specific Characters in the Group.

By

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Professor of Biology in the Presidency College, Madras.

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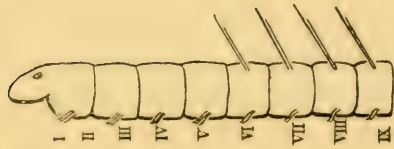
With Plates **XXVI** and **XXVII**.

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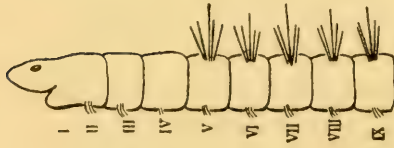
THESE notes were commenced at the instigation of Professor Lankester in 1882, to whom the late Mr. Thomas Bolton had forwarded specimens of a Naid which Professor Lankester identified as the *Nais littoralis* of O. F. Müller, together with another Annelid which I subsequently described as *Haplobranchus æstuarinus*.

Professor Lankester kindly placed in my hands all his drawings and notes relating to Naids; and those on *Nais* (*Paranais*) *littoralis* and *Pterostylarides macrochæta*, to each of which he had devoted considerable attention, are made use of in this paper.

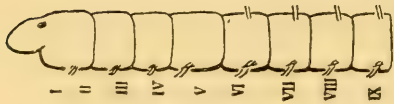
To Mr. Bolton I was indebted for a very large number of Naids collected in all parts of England, and I had intended to prepare a monograph on the British species of this group, but I left England with a series of scattered notes and half-finished drawings. Vejdovsky, in his magnificent 'System und Morphologie der Oligochaeten,' brought together his own researches and a summary of our knowledge



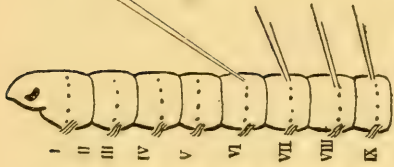
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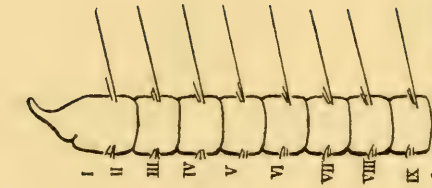
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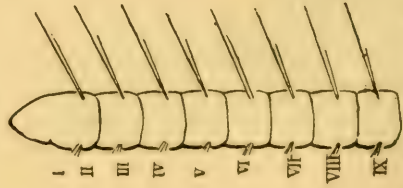
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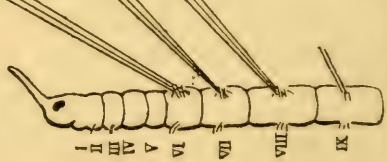
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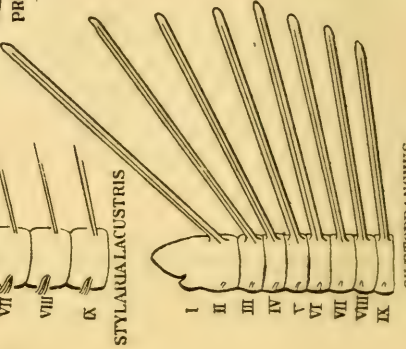
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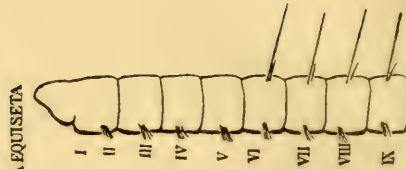
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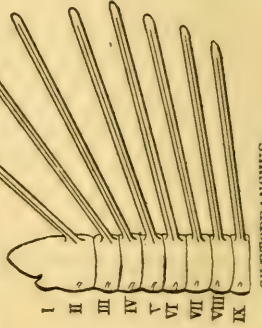
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CLUETROBRANCHIUS  
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of the group; and Mr. E. C. Bousfield<sup>1</sup> has still more recently in a valuable paper given a systematic account of the various species of the genus *Dero* at present known.

I am induced, even after this lapse of time, to publish some of my uncompleted notes, partly because no one has since described the new species which were discovered by Professor Lankester and myself,<sup>2</sup> and partly because no writer on the group has adequately dealt with the importance of the number of cephalized segments as a generic character.

A monograph of the British species is still a desideratum. I received from Mr. Bolton on several occasions specimens other than those herein described, which were certainly not referable to any existing species, but for which my notes are insufficient to warrant the creation of new species. It has frequently been pointed out, but very little stress has been laid upon the fact even by Vejdovsky,<sup>3</sup> that the dorsal setæ are often wanting in several of the anterior segments of the body, while ventral setæ are present in these segments. It is this character which chiefly marks what I term, at Professor Lankester's suggestion, a cephalization. There is almost always, if not always, a certain amount of cephalization in the Oligochæta; that is to say, there is in the anterior region a segment or number of segments which differ in their organization from the segments which follow, these latter being usually similarly developed throughout the remainder of the worm. This may be exhibited by peculiarities of the alimentary canal, the circulatory system, the arrangement of septa, the absence of nephridia from the most anterior segments, and so on. In most if not all Oligochæta there is a peristomial segment which is devoid of any setæ, and in many Nais the dorsal setæ are absent from three, four, or six of the

<sup>1</sup> 'Journ. Linn. Soc. Zoology,' vol. xx, 1887, p. 91.

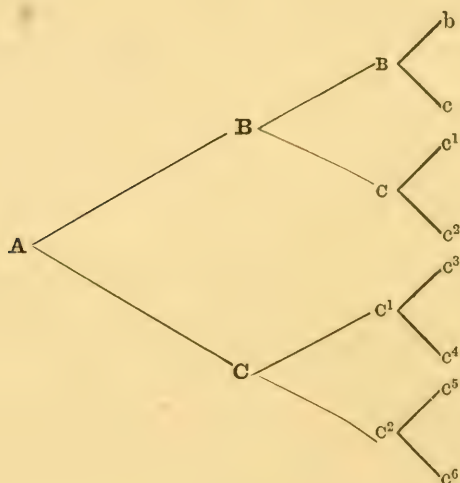
<sup>2</sup> Professor Lankester referred to *Pterostylarides macrochæta* (under the name of *Pterygonais macrochæta*) and exhibited my drawing of this worm, which is here published, at the meeting of the British Association at Southport in 1883.

<sup>3</sup> 'Oligochaeten,' 1884.

segments immediately following the peristomial segment; and, moreover, the number of segments thus modified (cephalized) appears to be constant in all the species of a genus.<sup>1</sup> The accompanying woodcut shows the various generic types.

The laws which govern gemmation or budding were worked out by Semper. I have added the following remarks upon the subject with the view of making this somewhat complicated matter clearer to English readers, in the hope that some microscopist may be induced to make further observations on the subject in species where we still lack information as to the budding individuals. There are many such, but there are an even greater number of species in which the sexual individuals have never been seen.

In the following diagram—



<sup>1</sup> The only recorded exception to this which I have found is given by Bousfield (l. c.), who states that in *D. furcata* there are only four segments destitute of dorsal setæ, while in all other species there are five such segments. I may, however, point out that *D. furcata* differs in another marked character from all other known species of *Dero* in the possession of "palpi," and should, I expect, be therefore placed in another genus.



**A** is a primary zoöid developed from the egg. **A** will give rise to two secondary zoöids, **B** and **C**, in the following manner:—after its  $n^{\text{th}}$  segment ( $n$  is a number characteristic probably of each species) a bud, **Z**, will form; this will divide into two regions,  $z$  and  $z'$ :  $z$  will consist of an indefinite number of segments, and form the tail of **B**; while  $z'$  will consist of a definite number of segments (a number characteristic of each genus), and will become the head of **C**.

**B** and **C** may now separate, or may remain joined together until two new budding regions have appeared.

In either case, after the  $n^{\text{th}}$  segment of **B** another bud, **Z**, may form, which will behave in the same way as the first bud behaved, and divide into  $z$  and  $z'$ ; and  $z$  will become the tail of a tertiary zoöid **B**; and  $z'$  the head of another tertiary zoöid,  $c$ ; and a similar process will take place after the  $n^{\text{th}}$  segment of **C**, giving rise to two other tertiary zoöids,  $c^1$  and  $c^2$ . The chain of four zoöids represented by the letters **B**,  $c$ ,  $c^1$ ,  $c^2$ , may now separate into two chains of two zoöids each,  $bc$  and  $c^1c^2$ , or so far as we know may remain joined together until, by the formation of four new budding regions, a chain of eight zoöids,  $b, c, c^1, c^2, c^3, c^4, c^5, c^6$ , has developed. As a matter of fact I believe that in no Naid do more than four zoöids hang together in a chain.

Among a mass of Naids where asexual reproduction was rife, specimens might occur which could be represented thus:—**A**, **B**, **C**, **BC**,  $B, c, c^1, c^2, BC, c^1c^2, b, c, c^1, c^2, c^3, c^4, c^5, c^6, bc, c^1c^2, c^3c^4, c^5c^6, bcc^1c^2, c^3c^4c^5c^6$ , &c.

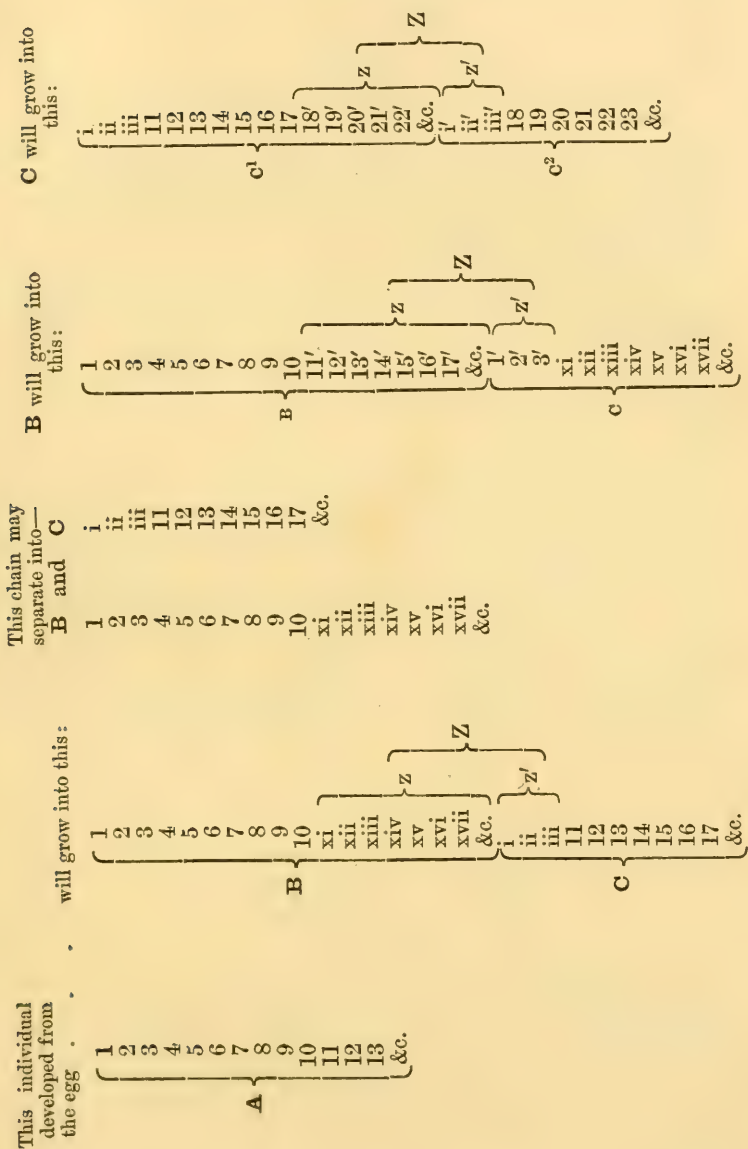
I am inclined to believe that the forms which do occur are **A**, **BC**,  $bc, c^1c^2, bcc^1c^2, bcc^1c^2, c^3c^4c^5c^6$ , &c.; and among these we may theoretically recognise three types:—(1) forms **A**, developed from the egg; (2) forms **BC**,  $bcc^1c^2, bcc^1c^2$ , &c., in which the original head end is retained; and (3) forms  $c^1c^2, c^3c^4c^5c^6$ , &c., in which the head is a budded region; and it is possible that there are forms **C**, which must be distinguished from  $c^1c^2, c^3c^4c^5c^6$ , &c., as they possess the tail end of a form **A**, while  $c^1c^2$  and  $c^3c^4c^5c^6$  must consist entirely of segments formed by budding.

I am not aware that any of these forms can be distinguished from one another by observation of their structure, but it would be most interesting to discover whether all or some of them only can become sexual individuals.

I believe that the number of segments in the sexual individual is constant for the species, while that in asexual individuals is indefinite; but that  $n$  is a number constant for the species, and that the number of segments in  $z'$  is constant for the genus,  $n$  and  $z'$  being used with the signification above described.

The segments represented by  $z'$  are referred to in the generic definitions as the cephalized segments: in most cases all these cephalized segments remain marked in the adult; in all cases the most anterior of them, the peristomial segment, is obvious, while the others are usually marked by the non-development of dorsal seta bundles; but in *Pristina*, at any rate, the bud shows that there are really seven cephalized segments (*i. e.*  $z'$  consists of seven segments), a fact which could not be ascertained by an inspection of the well-grown head region.

The following table represents six zoöids or chains of zoöids belonging to the same species, an imaginary species in which I have supposed  $n=10$ , and  $z'$  to consist of three segments; all the other letters have the signification above described. Instead of actually drawing the segments I have represented them by some numeral.



## SYSTEM OF THE NAIDOMORPHA.

The Naidomorpha are Oligochæta in which the central nervous system presents cerebral ganglia, pharyngeal commissures, and a ventral cord. The cerebral ganglia are always separated from the epiblast.

In addition to sexual, asexual reproduction by means of gemmation and subsequent fission occurs. A clitellum develops in sexual individuals at the breeding season.

The setæ are placed in four rows. They are capillary, spear-shaped, or crotchet-shaped.<sup>1</sup>

There are, as a rule, more than two setæ in each bundle.

A stomachal enlargement of the alimentary canal occurs in segment VII or VIII. They are all aquatic, living in fresh or sea water. Branchial processes may be present or absent.

To this definition must be added, when information is available, the position of the various generative organs. The position of the testes and ovaries given by Vejdovsky does not agree with my observations on *N. barbata* and *P. littoralis*.

According to Vejdovsky, the following genera form the family of Naidomorpha:—

*Aulophorus*, Schmarda.

*Dero*, Oken.

*Nais*, s. str., O. F. Müller.

*Bohemilla*, Vejdovsky.

*Ophidonais*, Gervais.

<sup>1</sup> I do not use these three terms to correspond exactly to Haar-borsten, Spalt-borsten, and Haken-borsten. The capillary setæ are the long hair-like setæ, which may be serrated as in *Bohemilla*; the crotchet-shaped setæ have the well-known *f*-shape, with the forked free extremity; while what I have called spear-shaped setæ are such setæ as present characters to some extent intermediate between those of the foregoing types. They may be straight with a sharp-pointed end, or straight and bifurcated at the end, or somewhat crook-shaped.



Slavina, Vejdovsky.  
 Stylaria, Ehrenberg.  
 Pristina, Ehrenberg.  
 Naidium, O. Schmidt.

I think it is desirable to add to this list *Pterostylarides*, Czerniavsky, for *P. parasita* (syn. *Stylaria parasita*, O. Schmidt) and *P. macrochæta*, sp. n., mihi; *Paranais*, Czerniavsky, for *P. littoralis* (syn. *N. littoralis*, O. F. Müller) and ? *P. uncinata*, and *Chætobranchus*, for *C. semperi*, mihi.

#### GENERA AND SPECIES OF NAIDOMORPHA.

##### *Aulophorus*, Schmarda.

1. *A. discocephalus*, Schmarda; Kingston, Jamaica.
2. *A. oxycephalus*, Schmarda; Galle, Ceylon.
3. *A. vagus*, Leidy; Philadelphia, N. America.

##### *Dero*, Oken (see woodcut).

1. *D. latissima*, Bousfield.
2. *D. Perrieri*, Bousfield.
3. *D. obtusa*, D'Udekem.
4. *D. limosa*, Leidy.
5. *D. philippinensis*, Semper.
6. *D. acuta*, Bousfield.
7. *D. furcata*, Oken (syn. *D. palpigera*, Grebnitzky;  
*D. rodriguezii*, Semper).

##### *Nais*, s. str., O. F. Müller (see woodcut).

Four cephalized segments in addition to the peristomial segment remain well marked in the fully grown head.

There is no prostomial tentacle.

Some or all of the dorsal setæ are capillary.

There are no branchial processes.

Eyes may be present or absent.

1. *N. elinguis*, O. F. Müller.

This species is common in England.

2. *N. barbata*, O. F. Müller.

This species is common in England. According to Semper<sup>1</sup> the budding zone appears after segment xvii in this species (i.e. *n*, as used on page 339, is equal to 17).

3. *N. josinæ*, Vejdovsky.

I have not seen this species.

4. *N. fusca*, Carter.

This species is recorded from India, but is not sufficiently characterised.

5. *N. scotica*, Johnston; England.

Not sufficiently characterised.

*Bohemilla*, Vejdovsky (see woodcut).

Three cephalized segments in addition to the peristomial segment remain well marked in the fully grown head.

There is no prostomial tentacle.

The capillary dorsal setæ are serrated.

There are no branchial processes.

Eyes are present in the only known species.

1. *B. comata*, Vejdovsky.

(Syn. *Nais hamata*, Timm.)

I received numerous specimens of *Bohemilla* from Mr. Bolton. It has not been, I believe, hitherto recorded from England. The specific characters agree very closely with those given by Vejdovsky and Timm. I never found a specimen without eyes; Vejdovsky makes no mention in his large book of their presence or absence, but Timm states that the eyes are not always present. I have noticed that the pigment in the alimentary canal commences in the region of the first dorsal seta bundle in this and many other species of *Nais*.

The nephridia are not present in all the segments. I observed nephridia in segments viii, xi, xvi, xviii, xx, and xxi, each with a funnel opening into the segment in front.

<sup>1</sup> 'Arb. zool. zoot. Institut. Wurzburg,' Bd. iv, 1877.

One of the most interesting features in the anatomy of this worm is the condition of the setæ in the cephalized segments. The first pair of ventral seta bundles, viz. those belonging to segment II, are larger than those in the two following segments. Those in segment IV are very small, and seldom contain more than two setæ—indeed, they are frequently altogether absent; but I have always found them in a newly budded head, so that when they are absent it is doubtless because they have dropped out. I have unfortunately no other notes with regard to the budding.

Ophidonais, Gervais (see woodcut).

Four cephalized segments in addition to the peristomial segment are well marked in the fully grown head.

There is no prostomial tentacle.

There are no capillary setæ.

There are no branchial processes.

Eyes are present in the only known species.

The only other known genus of Naids in which capillary setæ are absent is *Paranais*.

1. *O. serpentina*.

This species is well known in England; all the dorsal setæ are spear-shaped and straight.

*Slavina*, Vejdovsky (see woodcut).

Four cephalic segments in addition to the peristomial segment are well marked in the fully grown head.

There is no prostomial tentacle.

The dorsal setæ are capillary.

There is a girdle of papillæ in the middle of each segment.

1. *S. appendiculata*, Vejdovsky.

I received a few specimens of a species of *Slavina* from Mr. Bolton, but have no notes which enable me to offer an opinion as to the identity or otherwise of the English species with *S. appendiculata*, Vejdovsky.

According to Bousfield<sup>1</sup> the English species is identical with the *Nais lurida* of Timm, and he calls it *S. lurida*, and

<sup>1</sup> 'Journ. Linn. Soc. Zoology,' vol. xix, 1886, p. 264.

maintains that this species is not identical with *S. appendiculata*, as Vejdovsky believes it to be. As Stolc<sup>1</sup> has pointed out, this is very probable, and the difference is to be found in the arrangement of the tactile papillæ; but I agree with Stolc, that Bousfield has fallen into a great error in associating *Ophidonais serpentina* with the genus *Slavina*. The absence of capillary setæ distinctly marks *Ophidonais* as a separate genus.

*Stylaria*, Lamarek (see woodcut).

Four cephalized segments in addition to the peristomial segment are present in the bud, and remain well marked in the fully grown head.

The prostomial tentacle is very long.

The dorsal setæ are capillary.

There are no branchial processes.

Eyes are present in the only known species.

1. *S. lacustris*.

A full synonymy of this species, which is perhaps most widely known as *Nais proboscidea*, is given by Vejdovsky.

I have occasionally observed specimens in which dorsal setæ were present in the one or two most posteriorly placed of the usually cephalized segments. I can only suggest that these were abnormal individuals, but it is a matter worthy of further investigation. I described the process of budding in this worm at the meeting of the British Association at Aberdeen, in 1885. I quote here the substance of the note published in the report of that meeting. I have altered the numbering of the segments to make it accord with the system adopted in the present memoir, in which the peristomial segment is called segment 1.

“When budding is about to commence, a slight thickening of one of the septa which separate one cœlomic segment from another occurs. This thickening increases, the body-wall in the region thickens, and an actual bud is here formed. This

<sup>1</sup> ‘Zoologischer Anzeiger,’ vol. ix, 1886, p. 502.



new region elongates, and presents a solid appearance. The alimentary canal grows in this region, but the newly formed portion is at first unpigmented, and may still be detected at a much later period by its lighter colour. Its lumen remains, however, all the time, and a continuous line of fæcal matter may be observed (Pl. XXVII, fig. 9, *fæc.*). This budding region divides into two portions. The anterior portion develops numerous setæ, and gives rise to an indefinite number of segments which form the tail of the old worm. The posterior portion develops four pairs of ventral setæ, this development taking place from before backwards; and subsequently at its anterior region the peristomial segment and the characteristic proboscis are developed, and the two individuals separate. The budding region usually forms between segments XXVII and XXVIII, so that segment XXVIII becomes segment VI of the posterior daughter worm: the five anterior segments of this worm never present dorsal setæ."

What is described above as taking place is clearly shown in Pl. XXVII, figs. 7—9, which represent various stages of the process.

The division of the budding zone into a new tail on the one hand and a new head on the other was clearly stated by Semper,<sup>1</sup> whose paper on the subject I had not seen when writing the above.

It will be seen that, according to the nomenclature I have adopted,  $n = 26$ , and  $z'$  consists of five segments in this species:

Pterostylarides, Czerniavsky (see woodcut).

Four cephalized segments in addition to the peristomial segment are indicated in the fully grown worm. The two hinder of these are devoid of ventral as well as of dorsal setæ.

The prostomial tentacle is of medium length.

The dorsal setæ are capillary.

There are no branchial processes.

Eyes are present.

Vejdovsky associates this genus with Stylaria, but I think

<sup>1</sup> 'Arb. zool. zoot. Instit. Wurzburg,' Bd. iv, 1877.

that the peculiar character of the cephalization is a feature amply sufficient to warrant the separate genus.

*Pterostylarides* differs from all the other genera in the constant possession of cephalic segments other than the peristomial which are devoid of all setæ. It is, of course, an assumption that the region between the second and third seta-bearing segments does represent two segments. Vejdovsky in his "*Oligochæta*" assumes that this is the case; but I have not seen his paper on "*Thierische Organismen der Zusammenwässer von Prag, &c.*" In Pl. XXVI, fig. 1, which was drawn from nature, there is but the very slightest external indication of segments in this region, and I made no observations upon the septa.

As stated above, in *Bohemilla comata* the setæ in the most posterior cephalized segment are few in number and very small, and may disappear; when this occurs we have an undeniable instance of a cephalized segment devoid of all setæ. Such absence of all setæ is, I think, to be regarded as extreme cephalization.<sup>1</sup>

In the following description of *P. parasita* I have selected from Vejdovsky's description what seem the important points in distinguishing this species from *P. macrochæta*.

#### 1. *P. parasita*, O. Schmidt.

Prostomial tentacle of about the same length as the peristomial segment ("Mundsegment").

The eyes are on the dorsal side.

The ventral setæ of the second and third segments are curved and crotchet-shaped, and about a third longer, and with longer teeth than the ventral setæ of the other segments. There are five or six setæ in these bundles, and seven or eight in those of the other segments.

The setæ in the three most anterior dorsal bundles are

<sup>1</sup> There can be no doubt that *Chætogaster*, which in my opinion ought to be classed with the *Naidomorpha*, presents a region corresponding to two or even three imperfectly developed segments devoid of setæ between the first and second pairs of bristle bundles.—E. R. L.

capillary, and longer than the first five segments. There are twelve to fifteen in the bundle. The other dorsal setæ are capillary, and of about one fifth the length of the anterior ones.

2. *P. macrochæta*, mihi (Pl. XXVI, fig. 1).

The prostomial tentacle is much longer than the peristomial segment.

Eyes as in *P. parasita*.

The ventral seta bundles of the second and third segments consist of two to three setæ, and those of the other segments of two to five setæ.

The setæ in the three most anterior dorsal bundles are all capillary; two to five of these are very long, longer even than the long setæ in *P. parasita*, while the other four to six are shorter than the other dorsal setæ. The remaining dorsal seta bundles usually contain two setæ, one rather longer than the other.

I found no individuals with generative organs, and none exhibiting gemmation.

[The long setæ are frequently found thrown forward so as to partly encase and protect the head when the worm forms for itself a temporary tube. They are also used to strike the water in swimming.—E. R. L.]

*Paranais*, Czerniavsky (see woodcut).

Three cephalized segments in addition to the peristomial segment remain well marked in the fully grown head.

There is no prostomial tentacle.

There are no capillary setæ; all the setæ (dorsal as well as ventral) are crotchet-shaped.

There are no branchial processes.

Eyes may be present or absent.

1. *P. uncinata*, Oersted.

According to Czerniavsky the *Ophidonais uncinata* of Oersted belongs to the genus *Paranais*.

2. *P. littoralis*, O. F. Müller (Pls. XXVI and XXVII, figs. 2 to 6).

This species is by no means well known. It is recorded by

Oersted, and more recently mentioned by Czerniavsky, who founded the genus *Paranais* for it and the above-mentioned species. Czerniavsky found it in Abchasia.

Professor Lankester received specimens of this worm from the great mud-banks at Sheerness, at first through Mr. Bolton; but we afterwards obtained it in quantity from Mr. W. H. Shrubsole, who found it living in enormous numbers along with *Haplobranchus* and *Hemitubifex*. It is one of the few *Oligochæta* which are known to inhabit salt water.

I am able to give only such notes of its structure as were made at the time by Professor Lankester and myself.

The prostomium is blunt and rounded.

The setæ present an arrangement the main features of which are doubtless characteristic of the genus, and are given above in the generic definition.

Setæ are absent, as is universally the case, from the peristomial segment (segment 1).

In segments II, III, and IV, ventral seta bundles alone are present, while in all other segments dorsal and ventral seta bundles are present. The usual number of setæ to the bundles is as follows:

|           | Dorsal. |   |   |   |   |   |   | Ventral. |
|-----------|---------|---|---|---|---|---|---|----------|
| Segment I | .       | . | . | — | . | . | . | —        |
| „ II      | .       | . | . | — | . | . | . | 5        |
| „ III     | .       | . | . | — | . | . | . | 3        |
| „ IV      | .       | . | . | — | . | . | . | 3        |
| „ V       | .       | . | . | 3 | . | . | . | 3        |
| „ VI      | .       | . | . | 3 | . | . | . | 3        |

and so on. In the dorsal bundles there are occasionally two setæ only; and in the budding region there are, as a rule, in the early stages of the bud two setæ only in each bundle, one longer than the other.

The ordinary setæ vary but little in shape throughout the body.

In no other genus of Nais have all the dorsal setæ the crotchet shape, as is here the case (Pl. XXVII, fig. 6).

In *Ophidonais* none of the dorsal setæ are capillary, but are spear-shaped setæ, and unlike the ventral setæ of that genus.

In *P. littoralis* the setæ of the cephalized segments are



longer and thinner than those which follow, and the ventral setæ of the majority of the segments are a little thicker and shorter than the dorsal setæ (Pl. XXVII, fig. 5).

Modified genital setæ are present in sexual individuals. They are the ventral setæ of segment v. There is no evidence that they represent any interpolated segment. The ordinary setæ doubtless drop out from the ventral bundles in that segment during the breeding season, and are replaced by the modified genital setæ. There are usually three in the bundle. They are very stout, and longer than the ordinary setæ, and they possess a mere rudiment of the crotchet at the free extremity.

In the budding individual the arrangement of the setæ in a well-advanced bud is shown in Pl. XXVI, fig. 2. In the new tail the series of seta bundles are seen to fade away gradually as one passes backwards—that is to say, the most posterior bundle is the youngest. In the new head there are three pairs of ventral seta bundles, only the new head assumes from the first its adult character (compare this with *Pristina*).

The pharynx occupies about three segments, and at its sides and posterior to it occur two large glandular masses (Pls. XXVI, XXVII, figs. 2 and 3, *ph. gl.*), which are possibly groups of unicellular salivary glands. The “stomach” is well marked, and lies in segment VIII; the intestine is narrow in the following segment, and then widens out.

The dorsal and ventral vessels are clearly defined throughout the whole length of the worm. They communicate with one another by three pairs of branching lateral vessels in segments II to IV, and by three pairs of larger unbranched “hearts” in segments V to VII.

It is exceedingly interesting to note that in the newly budded regions and other regions of active growth, as in all tail regions, the dorsal and ventral vessels are joined by commissural vessels in every segment, and that this is therefore doubtless the primitive arrangement; and the suppression of the commissural vessels in all except certain anterior segments indicates a particular kind of cephalization (Pl. XXVI, fig. 2).

I was unable, even after repeated examination, to discover

any nephridia ; and their absence, if they are really absent, is a very remarkable character, which I should be glad to see verified.

I found numerous individuals in which the generative organs were well developed (Pl. XXVII, fig. 3).

The testes occur in segments VIII and IX, and the ovaries in segment X.

The spermathecae lie in segment V, and open near the modified genital setae, between segments V and VI.

I was able to make a few observations on the asexual reproduction.

The most complicated chain of zooids which I obtained exhibited two regions of active growth. Such a chain would be represented, according to the nomenclature which I adopt, thus :—BC, or BC, or  $c^1c^2$ , &c. According to the same nomenclature,  $n = 17$  in *P. littoralis*, and  $z'$  consists of four segments.

#### Pristina, Ehrenberg (see woodcut).

Seven cephalized segments—of which only one, the peristomial segment, is recognisable in the fully grown head region—may be distinguished in the newly budded head region.

The prostomial tentacle is short.

The dorsal setae are capillary or capillary and spear-shaped.

Branchial processes are absent.

Eyes are absent in all the known species.

#### 1. *P. longiseta*, Ehrenberg.

This species has been recently re-described by Vejdovsky. I have not seen it, nor has it, so far as I know, ever been recorded from England.

#### 2. *P. equiseta*, sp. n.

I found this species in great quantity in the Victoria regia tank in the gardens of the Royal Botanic Society at Regent's Park, London.

It differs from *P. longiseta* only in the non-development of the long setae to which that species owes its name.

It is very small, about 8 mm. long.

The majority of my specimens in which there were no generative organs, and which were not budding, consisted of eighteen to twenty-one segments.

I frequently found the ventral setæ of the fourth segment in non-budding individuals to be much larger and stouter than the other ventral setæ. These were probably modified genital setæ, but I never obtained specimens showing any further sexual developments. The dorsal setæ are all capillary and of similar lengths throughout the body. There are usually two in the bundle, one longer than the other; but those of the third segment are not extra long, as is the case in *P. longiseta*.

The "stomach" lies in segment VIII.

The blood is yellowish.

I observed a single pair only, of commissural vessels.

The most anterior nephridia lie in segment IX, and not in segment X, as in *P. longiseta*.

The cœlomic corpuscles are very large, and greenish in colour.

I observed numerous budding individuals, but in all cases there was only a single budding region in any particular specimen, from which I infer (though not with certainty) that as soon as a chain of two zooids is formed a separation takes place.

The budding takes place after segment XIII (i.e.  $n = 13$ ), and there are seven segments in the new head (i.e.  $z'$  consists of seven segments). The most anterior of these becomes the peristomial segment, and the remaining six develop dorsal and ventral setæ; so that in the adult they are indistinguishable, so far as the setæ are concerned, from those which follow.

### 3. *P. breviseta*, sp. n. (Pl. XXVII, figs. 11—15).

This is a species which I found in enormous numbers in one or two localities in Madras; and although I hope to describe it and other Indian Naids in detail at some future date, I refer to it now because, from observations made upon it, I have been enabled to verify the law of budding described in the foregoing portion of these notes.

*P. breviseta* grows to a larger size than the other species of *Pristina*, and contains a greater number of segments, and differs from them besides in two or three well-marked characters.

The dorsal setæ are of two kinds (Pl. XXVII, figs. 14 and 15), capillary setæ and spear-shaped setæ: the latter are peculiarly shaped, and are bifurcated at the distal extremity. (Compare with these Vejdovsky's figures of the dorsal setæ in *Naidium luteum*, and those figured by myself for *Chætobranchus*).

The capillary setæ are of about the same length throughout the body, except that in the second and third segments they are shorter (this is the character referred to by the name of the species).

Those of segment II are about half, and those of segment III about three quarters as long as those that follow.

The cœlomic corpuscles are black and very noticeable.

The most anterior nephridia are in segment IX.

I have not hitherto found sexual individuals. The budding takes place, as a rule, after segment XXII (i.e.  $n = 22$ ), and the newly budded head consists, as in *P. equiseta*, of seven segments (i.e.  $z'$  consists of seven segments in the genus *Pristina*). (See Pl. XXVII, fig. 12.) It has been largely the comparison of these two species of *Pristina* which has led me to believe that, as stated on p. 338,  $n$  is constant (with a few individual variations) for the species, while the number of segments in the new budded head is constant in the genus.

I give below a few examples, selected from the very large number of specimens in which I have counted the segments.

1. More than thirty segments, no budding.
2. More than forty segments, no budding.
3. More than forty-six segments, no budding.
4. More than fifty segments, with a budding zone after segment XX.
5. More than fifty-five segments, with a budding zone after segment XXII.
6. More than fifty-six segments, with a budding zone after segment XXII.



7. More than seventy-one segments, with a budding zone after segment XXII.
8. More than seventy-eight segments, with a budding zone after segment XXII, and another budding zone after the eighteenth segment beyond the new head of the anterior budding zone.

I say "more than" so many segments in all these cases because it is impossible to count the actual number of segments in the tail region.

*P. inequalis*, Ehrenberg, is insufficiently characterised, and *P. flagellum*, Leidy, does not belong, according to Vejdovsky, to the genus *Pristina*, but comes somewhere between *Dero* and *Aulophorus*.

*Naidium*, O. Schmidt (see woodcut).

One cephalized segment only recognisable in the fully grown worm, and no information as to the bud.

There is no prostomial tentacle.

The dorsal setæ are capillary and spear-shaped.

There are no branchial processes.

Eyes absent in the only known species.

1. *N. luteum*, O. Schmidt.

*Chætobranchus*, mihi (see woodcut).

One cephalized segment only, recognisable in the fully grown worm, and insufficient information as to the bud.

There is no prostomial tentacle.

The dorsal setæ are capillary and spear-shaped. Branchial processes enclosing some or all of the setæ of the dorsal bundles present in all the anterior segments, with the exception of the peristomial segment.

In the shape and arrangement of the setæ this genus very closely approaches *Naidium*, from which genus it differs in the remarkable branchial processes.

1. *C. semperi*, mihi.<sup>1</sup>

<sup>1</sup> 'Quart. Journ. Micr. Sci.,' vol. xxxi, 1890.

## DESCRIPTION OF PLATES XXVI &amp; XXVII,

Illustrating Professor Alfred Gibbs Bourne's "Notes on the Naidiform Oligochæta," &c.

## PLATE XXVI.

FIG. 1.—Surface view of *Pterostylarides macrochæta*. The segments are numbered 1—28. *m.* Mouth.

FIG. 2.—View of *Paranais littoralis*. The Arabic numerals indicate segments of the parent worm; the Roman numerals are attached to segments in the budding zone. *m.* Mouth. *ph.* Pharynx. *ph. gl.* Pharyngeal glands. *st.* Stomach. *d. v.* Dorsal vessel. *v. v.* Ventral vessel. *int.* Intestine. *n.* Nerve-cord.

## PLATE XXVII.

FIG. 3.—Anterior extremity of a sexual individual of *Paranais littoralis*. *ph. gl.* Pharyngeal glands. *gen. set.* Genital seta bundles. *sp.* Spermatheca. *t.* Testis. *ov.* Ovary.

FIG. 4.—Ventral seta from segment II, III, or IV, of *Paranais littoralis*.

FIG. 5.—Modified genital seta of *Paranais littoralis*.

FIG. 6.—Dorsal seta from *Paranais littoralis*.

FIGS. 7, 8, and 9.—Views of the budding zone in *Stylaria lacustris* in successive stages of growth. *Z.* Budding zone. *z.* Newly budded tail. *z'.* Newly budded head. *pr.* New prostomial tentacle. The Arabic numerals indicate segments of the parent worm; the Roman numerals indicate newly budded segments. *Fæc.* Fæces, showing how they pass through the newly budded region.

FIG. 10.—Enlarged view of the seta bundles of one side of the new head, from the same specimen as Fig. 9.

FIG. 11.—Diagram of the anterior extremity of *Pristina breviseta*.

FIG. 12.—Diagram of the budding zone of *Pristina breviseta*. Letters and numerals as in Fig. 9. The animal is drawn from the side to show the dorsal and ventral seta bundles.

FIG. 13.—Ventral seta of *Pristina breviseta*.

FIGS. 14 and 15.—Capillary and spear-shaped dorsal setæ of *Pristina breviseta*.

## On *Pelomyxa viridis*, sp. n., and on the Vesicular Nature of Protoplasm.

By

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With Plate XXVIII.

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My assistant, M.R.Ry. A. Sambasivan, B.A. (Madras), during the course of an exhaustive examination of the fauna of a small tank (pond) in the neighbourhood of the Presidency College discovered and drew my attention to numerous green "egg-like" bodies, of about  $\frac{1}{10}$  inch in diameter, which were to be seen on the surface of the finely divided mud.

These bodies proved upon examination to be Rhizopods belonging to the remarkable genus *Pelomyxa*, and forming a new species of that genus, but differing in some important particulars from all hitherto described Rhizopods. I propose to call the species *P. viridis*.

The species is peculiarly interesting not only on account of its great size (it is larger than any known form of the Lobosa), but also on account of the presence of chlorophyll and symbiotic bacteria,<sup>1</sup> and the assistance which this renders in the study of its protoplasm.

<sup>1</sup> The rod-like bodies here regarded as bacteria were described as crystals of unknown composition by Greeff in his account of *Pelomyxa palustris*. Leidy describes them as exhibiting transverse striations. The symbiosis of bacteria and amœboid Protozoa has a special importance at the present moment in connection with Metschnikoff's doctrine of Phagocytosis.

### Habitat and General Description.

I have only found *P. viridis* in one tank, and this a tank which has not been known to dry up for many years.

When the mud from this tank is placed in a dish with a little water and allowed to stand, several individuals of *P. viridis* may generally be found close to the surface of the mud; if these are removed and the mud stirred up and again allowed to stand, it is not usual to find many other specimens. So that I believe that this Rhizopod lives close to the surface of the mud. I have never seen it crawling on the sides of the dish or on any other object; nor have I ever seen it much spread out while in the mud, but when mounted on a slide with a cover-glass it readily spreads itself out and becomes very flat, and soon commences to exhibit active amœboid movements. Fig. 1 represents a mounted specimen, partially spread out and magnified about four diameters. It is difficult to convey any idea of the size to which such an organism may attain, as so much depends upon the extent to which it is spread out. I have seen specimens so much spread out that they would average as much as  $\frac{1}{3}$  inch in diameter. The specimens are of very varying sizes, but I have never found very small specimens; I have never, in fact, been able to find a specimen by the aid of the microscope which I had not previously picked out with the naked eye, although I have searched many slides of the mud for this purpose. It may be that small individuals are to be found at some other time of year, or it may be that I have not, in spite of my search, happened to alight upon a small specimen.

When viewed by reflected light individuals vary a good deal in colour, from the rich transparent green of a gelatinous lichen to that of a faded leaf. I presume that the chlorophyll undergoes some degeneration. I have never found any approach to the red colour found in *Euglena* or *Hæmatococcus*. There is often a whitish, more opaque-looking spot, such as has been shown in the middle of fig. 1. This is caused by an aggregation of sand particles, and it is often more clearly defined than in my figure. When seen by transmitted light



with a low power such regions naturally appear dark, but with polarised light they appear as bright spots, owing to the doubly refracting character of the sand particles. A number of these particles is always present, but at times their number is so enormous that there is almost as much sand as protoplasm. I have watched an individual so filled collect the greater number of these particles at one spot, and then simply pour them out from the protoplasm. An individual may thus get rid of hundreds of these particles in a minute or two, but the process of collecting them together preparatory to their extrusion is slow. I believe that there is a periodical wholesale extrusion of these particles. These particles have all the appearance of sand; they are of very varying size and of very irregular shape, and are insoluble in all ordinary reagents; they are crystalline in nature, and, as stated above, are doubly refracting.

The presence of these particles has not, I fancy, any special significance, they occur in great numbers in the mud and are taken in with the food; the animal, indeed, seems to exercise no discretion as to what it takes in. They become collected together as a mechanical result of slight movements, and when the animal makes some more extensive movements are thrown out in great numbers.

### Structure of the Protoplasm.

Viewed with an ordinary high power of the microscope the main mass of the organism appears of a green colour, while at the periphery, from time to time, perfectly colourless regions may be seen. One's first impression is that there is a green endoplasm and a colourless exoplasm (figs. 2 and 3). With the same magnifying power, in addition to the various protoplasmic contents described elsewhere, what appear to be granules may be recognised abundantly distributed through the greater mass of the protoplasm. The colourless peripheral regions above mentioned are usually devoid of these granules. The granules all prove upon further examination to be bacteria, and but for their presence the protoplasm itself would appear

absolutely non-granular. Exceedingly careful and repeated observations with a Powell and Lealand's  $\frac{1}{10}$  inch oil immersion objective, Abbé's sub-stage condenser, and Engelmann's dark chamber, which last is invaluable in the glare usually prevalent here, have enabled me to ascertain with great accuracy and clearness the real structure of the body substance.

The protoplasm is throughout a perfectly colourless and apparently homogeneous substance, and this substance, except in the peripherally placed portions which flow out from time to time to be sooner or later reabsorbed into the central mass, is densely packed with spherules of a semi-fluid stroma, impregnated with what I have ascertained to be chlorophyll, and to these chlorophyll-bearing spherules the green colour of the organism is due.

In these spherules we have not to do with chlorophyll-corpuscles, as they exhibit no phenomena of division, and are, moreover, more fluid than chlorophyll-corpuscles.

Their fluid character often becomes very evident after the death of the organism, when some of them may be usually observed to run together into larger masses. Where these spherules are packed closely together they assume the form of regular polyhedrons. They seem about as fluid as the stroma of human red blood-corpuscles. I have come to the conclusion that they correspond to the vacuoles or, as they are better termed, to distinguish them from other vacuoles, vesicles, which occur in so many specimens of protoplasm and give to such specimens a vesicular character.

The fact that they contain in *P. viridis* a substance impregnated with chlorophyll, a state of things hitherto unobserved, has enabled me to throw some new light upon their nature and upon the organization of the body substance.

The word protoplasm is used above in the sense in which Bütschli<sup>1</sup> uses the word plasma. It designates the substance which Leydig speaks of as the spongioplasma, as distinguished from hyaloplasma (chylema, Strasburger). In other words, I consider that the substance within the vesicles is to be

<sup>1</sup> Bronn, 'Protozoa,' p. 1392.

regarded as an entoplasmic product of the protoplasmic activity rather than as any portion of the protoplasm itself. The chlorophyllogenous stroma occupying the vesicles of *P. viridis* is no more entitled to be regarded as a portion of the protoplasm than are chlorophyll bodies or oil-globules, or any other structures ordinarily spoken of as protoplasmic contents. It is, of course, the presence of the chlorophyll which leads me to this conclusion. If I am correct in regarding these chlorophyllogenous structures as the homologues of the vesicles found in other protoplasms, the condition of *P. viridis* lends strong support to Bütschli's theory that the plasma is the substance which forms the envelopes of these vesicles only, or, as Bütschli has termed it, the substance forming the scaffolding of a honeycomb (Substanz des Wabengerüsts), and does not include their contents (Wabeninhalt).

The structure of *P. viridis* is, on the other hand, rather opposed to Bütschli's most recent views with regard to protoplasmic movements. If I understand rightly from the abstract available to me, Bütschli believes that a movement starts by the bursting of some vesicle, a phenomenon which would naturally escape observation in ordinary protoplasm, but of which, if it is constantly occurring, I should surely have seen something in a protoplasm whose vesicular contents are brightly coloured.

With regard to the homology which I have assumed to exist between the vesicles of ordinary protoplasm and those of *P. viridis* with their chlorophyllogenous contents, it is quite clear, in the first place, that these are the structures which render the protoplasm of *P. viridis* vesicular. There exist, it is true, in addition to them a number of vacuoles of varying sizes, but of these there are after all comparatively few, and if they alone were present the protoplasm could not be called vesicular. There is no evidence that these vacuoles contain any substance other than water.

We can undoubtedly distinguish in *P. viridis* between two varieties of non-contractile fluid-containing spaces (nicht contractile Flüssig-Keitsräume), the vacuoles containing water and the vesicles having chlorophyllogenous contents.

The vesicles vary very little in size, being always about  $\frac{1}{5000}$  inch in diameter.

The vacuoles, on the other hand, vary greatly in size, some being almost as small as the vesicles, while others attain five or six times that diameter.

No distinction has, as far as I am aware, been hitherto drawn between vacuole and vesicle in any so-called "vesicular" or "vacuolised" protoplasm.

Bütschli,<sup>1</sup> writing ten years ago, says of non-contractile vacuoles, "Seltener hingegen wird ihre Zahl so beträchtlich, dass das sie trennende Plasma nur noch ein Maschenwerk von Scheidewänden zwischen ihnen herstellt, das Plasma eine schaumige oder alveoläre Beschaffenheit annimmt. Ein derartiges Verhalten begegnet uns z. b. gewöhnlich bei *Pelomyxa*, auch bei gewissen Amöben tritt ähnliches mehr oder weniger deutlich hervor."

Of Bütschli's most recent views I can only judge from M. Yves Delage's<sup>2</sup> note on the models of protoplasm with which Bütschli has been recently experimenting, and if I understand rightly in, for instance, the soap and xylol model, the plasma (Bütschli) is represented by the soap and the chylema (Strasburger) by the xylol. So that the contents of both vacuole and vesicle would be termed chylema, but there is no word as to the possibility of the chylema being other than a single substance.

Now, in *P. viridis*, two distinct substances take the place of the xylol of Bütschli's model, the contents of the vacuoles and those of the vesicles, and were it not for the presence of the chlorophyll in one of them these would not be optically distinguishable one from the other.

Two species of *Pelomyxa* have been hitherto described, *P. palustris*, Greef,<sup>3</sup> and *P. villosa* (*Amœba villosa*), Leidy.<sup>4</sup> The protoplasm of both these species has been de-

<sup>1</sup> Bronn, 'Protozoa,' p. 101.

<sup>2</sup> 'Arch. Zool. Experimentale,' 1889.

<sup>3</sup> 'Arch. f. mikr. Anatomie,' Bd. x, 1874.

<sup>4</sup> 'Fresh-water Rhizopods of North America,' Washington, 1879.



scribed as highly vesicular, and Gruber,<sup>1</sup> in speaking of *P. villosa*, which he found in Germany, says, "Zunächst fallen die zahlreichen Flüssigkeitsvacuolen ins Auge, welcher den grössten Bestandtheil des Körpers ausmachen und demselben ein Schaumiges Aussehen verleihen. Diese Vacuolen, die von wechselnden Umfange Sind, liegen eingebettet in dem homogenen Plasma, welches mehr oder weniger feine Scheidewänder zwischen ihnen bildet, ähnlich eine Intercellularsubstanz zwischen den einzelnen Zellen eines Gewebes."

I think it probable that in these forms we have both vacuoles and vesicles, but as the vesicles are filled with colourless substance no distinction has been drawn between them. It will be interesting to re-examine these forms to ascertain if from the relative size and frequency any distinction can be drawn. The importance of the question lies in this, that the vesicles have probably an intimate relation to the structure of the protoplasm, and possibly to the production of amœboid movements; while the vacuoles vary from time to time in size and number, and have nothing to do with the ultimate structure of the protoplasm.

Fig. 6 represents a fragment of the protoplasm in a specimen which was deeply stained with osmic acid. At *x* is seen projecting at the surface a small portion of the hyaline protoplasm, devoid of all contents, stained brown and rendered granular by the osmic acid. The rest of the drawing shows the vesicles once occupied by the chlorophyllogenous substance bounded by the protoplasm. The chlorophyll has been dissolved, but whether any substance now remains in the vesicles I cannot determine; there is nothing which is stained by osmic acid. With all other fixing reagents which I have used the vesicular structure disappears.

The protoplasm of *P. viridis* appears then to be perfectly homogeneous, and small portions of it may at times be observed at the periphery of the organism free from all contents, but the great mass of it forms a mere scaffolding for the numerous vesicles, and is, moreover, densely packed with bacteria

<sup>1</sup> 'Zeit. f. w. Zool.,' Bd. xli, p. 189.

("crystals" of Greef), to say nothing of its various other contents. The vesicles contain a fluid substance impregnated with chlorophyll. The vesicles and the bacteria are to be regarded as bodies contained in the protoplasm, and the latter may flow out leaving all its contents behind. When the protoplasm does flow out in this way some of the bacteria soon follow, and may then be seen to start an active movement; and if the out-flow continues, the superficial vesicles leave the central mass and may be seen isolated in the hyaline protoplasm (fig. 5).

### Protoplasmic Movements, Pseudopodia.

Specimens of *P. viridis* will often remain for a long time absolutely quiescent; in such a condition the animal is, if not flattened by a cover-glass, fairly spherical in shape, and the green vesicles extend close up to the periphery. They always remain embedded in the protoplasm, so that they do not actually come to the surface; and, indeed, there is at the surface something more than the mere envelopes of the most superficially placed vesiculæ, or else the margin of an optical section would present a sinuous curve, the sinuosities of which corresponded to a row of vesicles, which is not the case.

In specimens where movements are taking place these movements are usually very active, and most so at the periphery, although they are by no means confined to that region. In this matter *P. viridis* agrees with other species of *Pelomyxa*.<sup>1</sup>

Long-continued movements often take place without the protrusion of any pseudopodia. Owing to its great size, *P. viridis*, and, indeed, other species of *Pelomyxa*, move about from place to place, at any rate while under observation, less frequently than smaller amoeboid organisms.

When the movements, which result neither in the protrusion of a well-marked pseudopodium nor in a translation of the organism, are taking place, the margin of an optical section consists of a hyaline layer, the movements in which have a wave-like, undulating appearance. It looks as though the

<sup>1</sup> Bronn, 'Protozoa,' p. 97, foot-note.

hyaline protoplasm protruded at some one spot, leaving all its contents behind, and then spread out laterally, while a sharply defined line seems to mark the original boundary; this line afterwards disappears, giving one at first the impression that the protoplasm, which has, so to speak, overflowed the original boundary but remained in contact with it, is separated from the rest of the protoplasm, except at the spot whence it actually protruded, by a sort of pellicle, which is subsequently dissolved. Gruber<sup>1</sup> has figured such an appearance for *P. villosa*, and says, "Wenn an einem ausgetretenen Protoplasmafortsatz die Vacuolen und die Körnchen einige Zeit durch eine scharfe Linie von der Sarkode getrennt bleiben, so beruht dies darauf dass das Pseudopodium durch Zusammenfliessen des Plasmas ueber diese Stelle hinentstand, und dass die feine peripherische Schicht von Protoplasma, welche vorher die Grenze gegen das umgebende Medium bildet, und welche jetzt von der Masse des Pseudopodiums umflossen wird noch eine Zeit lang erhalten bleibt;" and regards this as further evidence of the existence of a relatively hard cuticular layer or pellicle formed by the action of the water, a view which the same author put forward on a previous occasion.<sup>2</sup>

I cannot accept this interpretation of the facts. I do not believe that the hyaline protoplasm is protruded at one spot only, and that there is lateral displacement of its particles; the undulating appearance is chiefly caused, like other wave movements, by extrusions and retractions in a radial direction. The appearance of the boundary line may be explained by careful focussing, the line being at some slightly different level from the protruding protoplasm, and its disappearance being due to a subsequent protrusion at that particular level. I am not at all sure how far the existence of any such pellicle as Gruber describes is in accordance with the views as to the relation of protoplasm to imbibition water.<sup>3</sup>

If the density of the surrounding medium is increased, as

<sup>1</sup> 'Zeit. f. w. Zool.,' Bd. xli, 1884, pl. xiii, fig. 2, and p. 190.

<sup>2</sup> 'Zeit. f. w. Zool.,' Bd. xxxvi, 1882, pp. 457—469.

<sup>3</sup> See Engelmann on "Protoplasmic Movement," in this Journal, vol. xxiv, 1884, p. 390.

by the addition of a 1 to 2 per cent. solution of common salt, the protoplasm shrinks, owing to withdrawal of imbibition water. This might, of course, take place through the pellicle, but this is not probable, as such a layer must be impermeable, or difficultly permeable, to water, if it have the function of protecting the central protoplasm from the action of water; and, in any case, when a sudden shrinking took place, one might expect to see some crenation of the surface, such as is exhibited by a human red corpuscle when it is exposed to a medium denser than its own plasma. No such crenation takes place; but, on the other hand, some portions of the protoplasm usually stick to the cover-glass and become torn off, while some of the protoplasmic contents escape into the water and are carried away by the current.

I have at times observed a similar phenomenon when a long pseudopodium was quickly withdrawn. A specimen of *P. viridis*, which has been moving about under a cover-glass, habitually leaves some portions of itself sticking to the glass. Appearances, such as are shown in fig. 2, constantly occur where a pseudopodium is retreating, and are due to the sticking of the superficial protoplasm to the glass.

I have also constantly seen villi, such as Gruber<sup>1</sup> has figured for *P. villosa*, formed in the same manner.

Such phenomena seem to me to render the existence of any pellicle unlikely. Moreover, as will be described below, when a specimen of *P. viridis* is torn into pieces with needles the water seems to penetrate and cause almost instant disintegration. Now, if the protoplasm of these forms is in the habit of forming a pellicle directly any new surface is exposed to the water, why does it not do so in this case? It appears more probable that so long as the protoplasm is alive the amount of imbibition water which can be taken in is regulated, and that in the instance above cited the protoplasm is killed by a shock caused by the teasing, so that excess of water can at once penetrate and cause disintegration.

The pseudopodia are usually very blunt and lobose, and

<sup>1</sup> 'Zeit. f. w. Zool.,' Bd. xli, pl. xiii, fig. 3.



resemble those figured for *P. palustris*. When one of these large pseudopodia is about to be extruded the movements affect very deep-lying portions of the protoplasm; a wave-like bulging occurs, which gradually pushes out in a radial direction; an active current passes outward along its centre; all the protoplasmic contents in the neighbourhood are carried outwards and, having arrived at the extremity, pass back again in the peripheral portions of the pseudopodium, which thus narrows while it is elongating. The whole process gives one strongly the impression that the impulse for the extrusion is central and deep-lying in its origin. It is not that a small pseudopodium is gradually increased in size, but the mass of protoplasm extruded is actually greatest at first and then gradually diminishes. Even when fully extruded the backward peripheral currents still continue, especially in the proximal portion of the pseudopodium, which thus becomes flask-shaped, joined to the main portion of the organism by a narrow neck.

All this time no peripherally placed colourless layer appears, the vesicles and other protoplasmic contents being pushed on up to the surface; but when this pseudopodium commences to retreat the contents pass backward faster than the protoplasm itself, and a colourless superficial layer is left. Fig. 3 represents the distal extremity of a pseudopodium which has just commenced to retreat. If this withdrawal is rapidly continued some of the colourless protoplasm will be left sticking to the glass.

Besides these large pseudopodia, small ones are often found which seem to take their origin in the superficial layer. In these latter the hyaline protoplasm is first protruded, and into this a number of the bacteria appear to burst out, followed almost at once by some of the vesicles, which often separate a good deal from one another and appear as so many isolated spherical green droplets; these are sometimes followed by the other protoplasmic contents (fig. 5). These pseudopodia are never prolonged to any extent.

The rate at which the large pseudopodia are protruded is

considerable. I have found it to be about 0.75 mm. a minute. Engelmann<sup>1</sup> states that a velocity of 0.5 mm. a minute is sometimes attained by an *Amœba*, and may be considered as exceptionally rapid. I have made my observations at the ordinary temperature of the room; but this is about 30° C. at this time of year, and is thus not far below the optimum temperature for protoplasmic movement.

### Nuclei.

The number of nuclei present is enormous. I have endeavoured to estimate the number on stained preparations. I have made these preparations in various ways; one of the most satisfactory is to fix and harden on the slide with chromic acid (2 per cent. solution), followed by water and alcohol of increasing strengths; stain with picro-carmin, wash, and again treat with alcohol of increasing strengths; dehydrate, clarify, and mount in Canada balsam.

I put down the number of nuclei present in a large individual at 10,000.

They vary a little in size, but average, when living, about 0.03 mm. in diameter.

It has struck me that there may be some connection between the bulk of nuclear matter and the bulk of protoplasm connected with it.

I calculate that in *P. viridis* all the nuclei taken together occupy  $\frac{1}{60}$  of the total bulk of the organism. I have not many data at hand for comparison with this calculation, but in a mammalian ovarian ovum the nucleus occupies about  $\frac{1}{60}$  of the bulk of the ovum. So that 10,000 nuclei in *P. viridis* give relatively about the same bulk of nuclear matter as is found in a mammalian ovum. The nuclei closely resemble the nuclei of other species of *Pelomyxa*.

In a fresh condition they are spherical, and consist of a nuclear membrane containing a perfectly clear and transparent fluid nuclear substance, in which are seen from nine to twelve highly refracting spherical nucleoli (fig. 7).

<sup>1</sup> L. c., p. 379.

I have never seen these enlarged or containing anything like a vacuole, a condition described by Greef for *P. palustris*; nor have I ever seen anything in the protoplasm of *P. viridis* resembling the "glanz-körper" of that author, or which might be nucleoli escaped from a nucleus.

I have frequently seen nuclei extruded from the protoplasm, usually along with food débris or sand particles; when so extruded they remain for some time unacted upon by the water, but in time the water seems to penetrate the nuclear membrane, and the nucleoli exhibit Brownian movements, and after some time come out into the water (fig. 9); but although I have watched them for a long time I have not observed them to undergo any change.

I have been unable by the use of any preservative or staining process to make these nucleoli behave as does the single nucleolus of so many *Amœbæ*. This latter when treated with osmic, chromic, or picric acids, and subsequently with picrocarmine or alum carmine, stains deeply, while the nuclear substance remains clear and transparent, almost unstained. But when any one of these acids or even acetic acid is allowed to act upon the nuclei of *P. viridis*, the nuclear substance becomes opaque and granular, and the nucleoli disappear from view on account of the now opaque character of the nuclear substance. When stained, either by osmic acid or by picrocarmine or alum carmine (preceded by fixing and hardening reagents), and mounted in Canada balsam, they appear as quite different bodies from the fresh nuclei. To such an extent is this the case, that until I had actually watched a single nucleus throughout the entire process I was not satisfied that the bodies which I had taken to be nuclei in the fresh state were actually such bodies. What really happens is, according to the views of Pfitzner and others, that the chromatin substance, which is something distinct altogether from the nucleoli, is brought into view by the staining process, and obscures to a certain extent the nucleoli. The nucleus stains diffusely all over, while very numerous minute granules appear to stain more deeply.

I have even after most careful examination of specimens, mounted whole and of sections, been unable to find any cases of nuclear division.

### Other Protoplasmic Contents.

I have already spoken of the chlorophyllogenous substance, the permanent vacuoles, and the sand particles.

In addition to these, I have observed in many individuals small globular refractive bodies, about  $\frac{1}{1600}$  inch in diameter, which, from the fact that they are blackened by osmic acid, I suspect to be of a fatty nature. They are much more numerous at some times than at others.

I have on two or three occasions found all the specimens then examined crowded with large (about  $\frac{1}{200}$  inch in diameter) disc or lens-shaped, fairly transparent, but not highly refracting bodies, which, from the fact that all these specimens had been feeding on some special substance, I believe to be nutritive bodies of some kind. I have very little doubt from the appearance of the *débris* that the food on these occasions had been *Spirogyra* filaments, but I was never fortunate enough to see them in the protoplasm in an undigested state. The protoplasm in all these individuals was simply crammed with the food *débris*. It is probable that these specimens had got hold of some *Spirogyra*, of which there was a little on the surface of the mud, and that it had proved a very easily digestible food, and that a great deal of nutriment had become stored up in the protoplasm. The bodies under discussion stain a rich deep colour with iodine, but do not lose the colour on warming. They are probably some amyloid substance.

*P. viridis* takes in plenty of solid food of all kinds, *Daphniæ*, *Ostracods*, *Diatoms*, *Naiads*, &c. I have also found specimens which had taken in two or three or more plants of what I believe to be *Wolffia arrhiza* (one of the *Lemnaceæ*), and which swarms in most of the tanks in Madras, and is by-the-by often eaten, curiously enough, in enormous quantities by the tank frogs.



The food sometimes lies in food vacuoles, while at other times the surface of the food particles is actually in contact with the protoplasm.

Occasionally I have observed, when a large piece has lain in a large vacuole, pseudopodia protruded from the vacuolar wall and reflected over the surface of the food particle.

### Physiology.

I have tried but without success to demonstrate the activity of the chlorophyll. The animal does not readily allow of much experiment, as it almost always dies after any manipulation. Treatment with iodine has, moreover, failed to demonstrate the presence of starch. The enormous number of the symbiotic bacteria is correlated doubtless with the activity of the chlorophyll.

I have tried by putting them together under a cover-glass to get two individuals to fuse together, but without success. On the other hand, I have often observed two pseudopodia of the same individual form and fuse together at their distal extremities, so as to leave for a time a hole right through the animal. Further, I have several times cut a specimen in half with a sharp scalpel, and kept the two halves living for some time, and by placing the two halves close together have induced them to fuse together again.

I have also teased specimens into fragments with a pair of needles, but in that case the fragments have at once commenced to die.

No information which we at present possess seems to me to explain in any way why the halves of a divided individual or the peripheral extremities of long pseudopodia protruded by any one individual should freely reunite, while separate individuals will do nothing of the kind.

Gulliver's Views.—Since writing the above my attention has been drawn to a note on the minute structure of *Pelomyxa palustris* by Dr. G. Gulliver.<sup>1</sup> The author believes, in the first place, that the exoplasm is permanently differentiated

<sup>1</sup> 'Journal of the Royal Microscopical Society,' 1888, p. 11.

from the endoplasm; and in the second place, that the "endoplasm" is composed of a number of cells. Both these are very startling views, and while I feel sure that the conclusions are erroneous, I have observed certain phenomena which may have given rise to them.

Gulliver writes, "In the process of hardening this layer (the exoplasm) readily separates from the subjacent softer endoplasm." I have noticed in hardening specimens in a watch-glass for section-cutting, and also under the cover-glass for subsequent staining and mounting as whole preparations, that the peripheral portion which is first attacked by the hardening reagent often becomes separated from the still living and shrinking central mass, another layer is then attacked, and so the whole structure may harden in irregular layers. The hardened portions break away with extreme readiness from the unhardened ones.

Very curious appearances are sometimes thus produced, but I am convinced that the phenomenon is a mere accident in the hardening process. The examination of living specimens can leave no doubt as to the absence of any permanent differentiation of the body substance into exoplasm and endoplasm. With regard to the structures which Gulliver takes to be cells building up the "endoplasm," and which Lankester has suggested may be swarm-spores, I have formed a less definite opinion. In a living specimen one can see no such structure, but in hardened specimens one does undoubtedly find small rounded portions of the protoplasm lying in and yet separated from the rest of the protoplasm. These bodies have, however, no definite and constant characters, they differ considerably in size, sometimes include one nucleus, sometimes more than one, and frequently none at all. It is difficult to detail the precise appearances which lead me to the conclusion; but I am much inclined to think that we have here merely an accidental rounding off of portions of the protoplasm, which takes place during the hardening process. I have seen similar phenomena in specimens which have died from having been kept mounted for a long time under a cover-glass. The body

substance in these large *Pelomyxæ* forms a considerable mass, and this breaks up very readily after death into small droplets.

I find nothing in the structure of *P. viridis* which would lead one to suppose that these organisms have any affinities, other than those usually assigned to them. *Pelomyxa* belongs, as Bütschli says, to the family *Amœbæa lobosa*.

In spite of repeated endeavours I am unable to throw any light upon the reproductive processes which may obtain in *Pelomyxa*.

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## EXPLANATION OF PLATE XXVIII,

### Illustrating Professor Alfred Gibbs Bourne's paper on "Pelomyxa viridis."

FIG. 1.—An entire specimen, partially spread out under a cover-glass, magnified about four diameters. In the left-hand lower corner is a *Daphnia* shell, which the animal had just extruded. The opacity of the central region is caused by a great accumulation of mud or sand particles.

FIG. 2.—Extremity of a blunt pseudopodium, which is retreating. Small droplets of the colourless non-granular protoplasm are being left sticking to the slide; some of these will become detached as the pseudopodium retreats further. The magnification is not sufficient to allow of the nuclei and vesicles being distinguished, but a few of the larger vacuoles, *v. v.*, are seen.

FIG. 3.—A long thin pseudopodium in the act of retreating, drawn to an even smaller scale than the preceding figure.

FIG. 4.—Extremity of a blunt pseudopodium, more highly magnified. The existence of the peripheral layer of colourless protoplasm indicates that the pseudopodium is not being actively protruded, while its small extent and comparative regularity shows that the pseudopodium is not being rapidly withdrawn. *n. n.* Nuclei. *f. v.* Food vacuole, containing food débris. *s.* Naid seta. *m. m.* Sand or mud particles. *v. v.* Vacuoles. The small circles which are shown all over the figure, except in the colourless peripheral portion, represent the vesicles with their chlorophyllogenous contents. In places

where, as over the food vacuole, marked *f. v.*, there is only a single layer of these vesicles, the green coloration is very faint.

FIG. 5.—Portion of the periphery of an individual where there has just been a slight outflow of colourless protoplasm, and which will be withdrawn without the protrusion of any pseudopodium. The bacteria (? crystals) seen coming out into the protruded protoplasm exhibit active (? Brownian) movements. The green bodies represent the vesicles; two of these are seen coming out into the protruded protoplasm.

FIG. 6.—View of a region precisely similar to that drawn in the preceding figure, in an individual which has been treated with osmic acid. The colourless protoplasm has stained; while the vesicular contents, now no longer green, are not to be seen. It is seldom that the vesicular structure is so well seen; usually, upon the application of reagents, the vesicles seem to collapse, and so no such network as is here shown can be seen.

FIG. 7.—View of a small portion of the central region of an individual, showing the same structures as in Fig. 4, with the addition of the bacteria. Three nuclei are drawn with their contained nucleoli.

FIG. 8.—A nucleus with an unusually large number of nucleoli.

FIG. 9.—A nucleus which has been extruded with food debris, and which has swollen under the action of the water, while its nucleoli have burst out.

N.B.—Figs. 5—9 are drawn to about the same scale as the line A B at the bottom of the plate, which represents  $\frac{8}{1000}$  of an inch.



## The Medusæ of *Millepora murrayi* and the Gonophores of *Allopora* and *Distichopora*.

By

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With Plates XXIX and XXX.

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### I. THE MEDUSÆ OF MILLEPORA MURRAYI.

IN 1884, Quelch (11), while examining the structure of the hard parts of a new species of *Millepora* (*M. murrayi*), discovered a number of small cavities which he supposed to be the receptacles of the ova or embryos like the ampullæ of the *Stylasteridæ*.

Professor Haddon has recently placed in my hands some excellently preserved specimens of a species of *Millepora* that he collected on the reefs of one of the islands in Torres Straits. This species seems to be closely allied to Quelch's *Millepora murrayi*, but the identification is a matter of some difficulty, as the pieces at my disposal are small.

On making a series of sections through a portion of a decalcified branch I discovered a number of medusiform structures, each bearing a large saucer-shaped spermarium. They are situated immediately beneath the surface, and covered by an operculum of modified ectoderm cells.

Sections made by von Koch's method of grinding hard and soft parts together in solid Canada balsam show further that these medusæ exactly fit into the ampullar cavities of the skeleton, and form the only explanation of their presence.

The eggs of this species are, as in *Millepora plicata* (6), very small and contain no yolk, and I have seen no embryos and no parasites that could cause or fit into these cavities. Quelch's ampullæ, then, are the cavities that contain male medusæ.

**The Structure of the Medusæ.**—The medusæ may be found in all stages of development in the different parts of the same branch. They are very irregularly distributed, and it is difficult at present for me to give any hints to guide naturalists in the search for them. They are never found, so far as my experience goes, close to the free extremities of the branches. In my specimens they were found in greatest abundance at a distance of three quarters of an inch to one inch from the free extremity, but a few specimens were found quite close to the attached base of the colony. Some branches appear to be devoid of them.

All the stages of development may be found with care and patience, but the stage represented in fig. 10 is the most frequent in my preparations.

A central MANUBRIUM (*Man.*) hangs in the sub-umbrella cavity bearing the large spermarium (*Sperm.*). It is composed of irregular endodermal cells, and contains a considerable cavity continuous with the cavity of the subjacent cœnosarcal canals.

The SPERMARIUM appears to be double in section, but is really saucer-shaped. It contains a large number of spherical spermoblasts lying in a homogeneous fluid (?). It is covered by a very thin coat of flattened ectoderm cells continuous with the inner ectodermic lining of the umbrella.

The UMBRELLA is composed of three layers: a median layer of solid endoderm continuous with the endoderm of the manubrium, and an inner and outer sheath of ectoderm continuous with one another at the free rim of the umbrella.

The inner sheath of ectoderm is, as mentioned above, continuous at its proximal side with the thin coat of ectoderm covering the spermarium. The outer sheath is continuous with a sheath of ectoderm (*Gon.*) lining the cavity of the

ampulla; and this again is continuous with the superficial ectoderm of the colony.

At the margin of the umbrella both ectoderm and endoderm are thicker than they are elsewhere, and the medusa presents in consequence a thickened rim at its free border. There are no radial or ring canals. In medusæ at this stage no cavity is apparent between the outer wall of the umbrella and the ectoderm lining the ampulla.

Above the codonostome (i. e. mouth of the umbrella) there is an operculum (*op.*) of flattened ectoderm cells continuous with the superficial ectoderm and the ectoderm lining the cavity of the ampulla, which completely closes the gonangium.

Different Forms of the Medusa.—The spermarium varies immensely in size. Sometimes it is simply a thickened ring round the manubrium, sometimes it nearly fills the cavity of the umbrella. In consequence perhaps of this variation in the size of the spermarium, the appearance of the manubrium varies. In fig. 10 the manubrium is a large well-developed structure with a considerable lumen. In fig. 9, which represents a younger stage, there is no manubrium at all apparent, but the spermarium simply rests on an irregular mass of vacuolated endoderm cells. Many intermediate conditions between these two extremes may be observed. Further, the condition of the endoderm of the manubrium presents many variations. In some cases the cell outlines are well marked, and the nuclei regular in position and spherical in shape. In other cases the endoderm is a loose vacuolated tissue in which no cell outlines can be distinguished, and the nuclei are irregular in shape and scattered through the spongy substance of the tissue.

It is not my purpose to offer in this place any explanation of these appearances. I wish merely to call attention to them before passing on to other matters.

Development of the Medusa.—The medusa of *Millepora* is a transformed zooid. It is not a zooid specially modified from its first appearance to bear the spermarium, but

an ordinary zooid of the colony changed into a medusa after the migration of spermospheres into its ectoderm, and subsequent development there.

The evidence that supports this statement rests upon a number of observed facts, that for convenience' sake may be arranged under the following heads:

1. The various stages in the transformation of the zooids into medusæ that can be observed in sections of the decalcified corallum.

2. The absence of any structure that can be compared to the ectodermic invagination, called the entocodon or glockenkern, that characterises the early stages in the development of the medusa of the Hydroidea.

3. The position of the medusæ in the colony.

4. The presence of large nematocysts in the superficial ectoderm above the younger forms of medusæ.

1. The most important of these, and the only one upon which much stress can be laid, is the first. The others afford the necessary confirmation.

The earliest recognisable forms of the sperm mother-cells are found in the canals in the immediate neighbourhood of the zooids (*Sperm. S<sub>2</sub>*, fig. 1). They migrate from this position into the ectoderm of the zooids, where they collect together to form a spermarium.

That the sperm mother-cells do actually migrate from the germinal epithelium into the zooids seems to me to admit of no doubt. The youngest stages of the germ-cells are never found in any part of the zooids, and the youngest stages of the zooids never bear either germ-cells or spermoblasts. These two observations prove, firstly, that the germ-cells do not arise in fully developed zooids; and secondly, that new zooids or medusæ are not formed at the localities in the canals where the germ-cells arise. They must, therefore, move from the position where they are first developed to the position they occupy in the zooid.

In a few cases I have seen two or three spermospheres (*Sperm. S<sub>2</sub>*, fig. 1), or aggregations of spermospheres, lying



separately in the ectoderm of the zooids, but in the majority of cases there is but a single cluster or aggregation (figs. 2, 3, and 4). The largest and most fully developed of these lie at the apex of the zooids (figs. 5, 6, and 7).

The conclusions from these facts seem to be that the germ-cells developing in the canals until they reach the stage corresponding to the sperm-morula or spermosphere migrate towards the zooids, fusing into aggregations as they do so. Having reached the zooids they take up a position between the ectoderm and endoderm of their apices, and continue there the later stages of their development.

The spermospheres are most frequently found in the dactylozooids, but in a few cases I have found them in gastrozooids (fig. 3). They have probably no preference for either the one form or the other; but they are found more frequently in the dactylozooids, partly because these forms are more numerous, and partly because the gastrozooids are usually more remote from the larger cœnosarcal canals.

The spermarium having been formed at the apex of the zooid certain noticeable changes take place. In the first place by a thickening of the ectoderm the pore becomes narrowed (figs. 5, 6, and 7). The tentacles become flattened out, and the nematocysts disappear. The spermarium sinks into a cup-shaped receptacle on the summit of the zooid, and the endoderm of the edge of the cup grows out, pushing before it the ectoderm.

These changes are represented in the two figs. 6 and 7. In the next stage the cup-shaped receptacle of the spermarium has grown out into a bell-shaped structure (fig. 8). The spermarium is much larger in size, and the pore is completely closed by ectoderm. In the later stages (figs. 9, 10, and 11) the following changes may be noted. The operculum is formed, shutting off all access from the cavity of the gonangium to the exterior. The walls of the bell-shaped outgrowth become considerably attenuated, and lie close against the ectodermic wall of the ampulla. The manubrium is formed probably by

a regeneration of the endodermic tissue and its growth into the centre of the spermarium.

In the last stage I have observed the medusa is completely separated from the canal system, and lies freely within the cavity of the ampulla. The walls of the umbrella, except at the margin, are extremely thin. The manubrial endoderm contains a closed cavity (fig. 11). This stage is probably the last that occurs before the embryo escapes from the corallum. There are no nematocysts developed on the thickened margin of the umbrella, there are no sensory bodies, there is no velum, and no mouth.

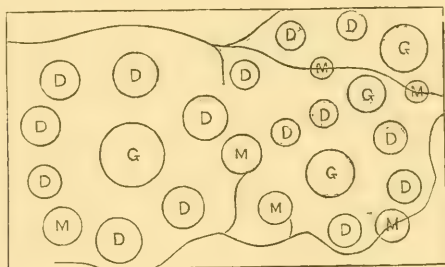
2. In the development of the medusæ of *Millepora* that has just been described there is no structure formed at any time that can be compared with the inner fold of ectoderm or "glockenkern" that forms the walls of the sub-umbrella cavity of the medusa of the Hydroidea. Had such a structure been found, there might have been some ground for supposing that this medusa is a bud that grows out of the degenerated tissues of a zooid. The medusæ of *Millepora* are, however, certainly not formed by budding from the zooid in the sense that the medusæ of such a form as *Corymorpha* are budded from the hydranth.

3. The diagrammatic figures that are frequently given of zooids of *Millepora*, representing a centrally placed gastrozooid in a complete circle of dactylozooids, is perfectly correct for some species of *Millepora* and the younger branches of others.

In *M. murrayi* the zooids are scattered over the older parts of the corallum in an irregular manner. The circular systems can be made out, but over and above the zooids in their regular circles there are both gastrozooids and dactylozooids scattered irregularly within and between the circles (cf. Quelch [11], p. 192).

The medusæ occur both in the regular circles and irregularly between them, as may be seen by reference to Woodcut 1. When a piece of *Millepora* is decalcified and cleared in oil of cloves or turpentine, and examined with a low power of the

microscope, the arrangement of the zooids, medusæ, and cœnosarcal canals can be very readily observed. The figure I have given was drawn, by the help of the camera, from such a preparation. The larger canals to which I have referred above form a wide-meshed network immediately below the surface. Each mesh is an irregular polygonal figure embracing the



WOODCUT 1.—Diagram of the arrangement of the zooids and medusæ of *Millepora murrayi*. G. Gastrozooids. D. Dactylozooids. M. Medusæ. The larger canals are represented by irregular black lines.

whole of one circular system of zooids. The medusæ are always found either upon or quite close to these large canals, and thus they are sometimes without the circles, and sometimes in a position corresponding to that of a dactylozooid of the regular circle.

The position of the medusæ in the colony cannot be used as an argument against my statement of their origin; in fact, whatever bearing it may have is in its favour.

4. When a decalcified specimen of *Millepora* is examined from above, a cluster of large nematocysts may be seen at the mouths of the gastropores and dactylopores. They may also be seen in sections (figs. 1, 2, 6, 7, *Nemat.*). When the medusa is formed and the pore closed by the operculum these large nematocysts can be of little or no service, so they are shot and no new ones take their place. In the figures of the sections through the older medusæ (9, 10, 11) the reader will notice that none of these large nematocysts are to be seen. Where the operculum is not completely formed (fig. 8),

although the zooid has to all appearance changed into a medusa, one or two of these large nematocysts remain.

## II. THE MALE GONOPHORES OF ALLOPORA AND DISTICHOPORA.

1. *Distichopora*.—The male gonophores of *Distichopora* may be seen in clusters on the branches of the male stocks. They are small whitish bodies lying in the ampullæ of the cœnosteum, and covered by a very thin semi-transparent wall of lime and cœnosarcal canals.

An examination of a series of sections through one of these branches shows that the male gonophores are found only in these superficial clusters (fig. 12). They are never found deeply seated in the cœnosteum, nor in other places than those indicated by external appearances.

One, two, or even three gonophores, in different stages of development, springing from a diverticulum of the cœnosarcal canal system, may occupy each ampulla.

A ripe male gonophore (fig. 14<sub>1</sub>) is a spherical, oval, or pear-shaped body, with an endodermal cell mass, representing the trophodisc on the side turned towards the axis, and a short conical or tubular seminal duct on the side turned towards the periphery. The sheath of the gonophore seems to be a simple layer of flattened ectoderm; but I am persuaded, after the examination of a great many sections and the study of the development, that two layers are represented, the inner or endodermal layer being extremely attenuated and devoid of nuclei.

When a very young bud is examined with a high power (figs. 13<sub>2</sub>, 14<sub>3</sub>, 15), the rudiment of the spermarium may be seen to be a homogeneous mass of protoplasm, containing a number of large spherical nuclei. It occupies a position apparently between the ectoderm and endoderm of the bud. As the spermarium increases in size the endoderm becomes cup-shaped in the bud, and the margins of the cup are produced into a very thin sheath between the ectoderm and the



spermarium (fig. 16). At the peripheral pole of buds that are about half-way developed there is a thickening of the two sheaths of the gonophores, cell outlines are well marked, and the cells are nucleated (figs. 14 and 16). In this way the first rudiment of the seminal duct is formed. The two layers are from their first appearance quite distinct from one another, and there is never any indication that the two cell layers are formed by a splitting of the ectoderm. Just before the spermarium becomes mature the ectoderm, and subsequently the endoderm, are folded to form a conical cap, and this subsequently pushes through the superficial covering of the gonangium to form the seminal duct to the exterior (fig. 18).

Meanwhile, changes have taken place in the endoderm at the base, i. e. on the axial side of the bud. In the early stages of the bud there is a wide lumen in the endoderm, the cells are cubical in shape, and their outlines well marked; in the later stages the lumen becomes obliterated, the cells lose their distinct outline, and the endoderm degenerates into an irregular mass of tissue, with scattered nuclei (figs. 13, 14, 15, 16).

2. In *Allopora* (fig. 19) the male gonophores are scattered irregularly in the corallum, and lie at such a distance from the surface that there is no trace of their existence externally. I have been able to find them only in the old thick branches. I cannot say for certain whether *Allopora* is hermaphrodite or dioecious. The specimens at my disposal consisted of a number of fragments in a bottle, and I found on the smaller and younger branches numerous female gonophores, and on the thicker and older branches numerous male gonophores; but I have not found both sexes on the same branches. I have no information whether the older and younger branches in the bottle are fragments of the same colony. If *Allopora* is not dioecious, then it is probably protogynous, like *Millepora*, the female sexual cells being formed first in the younger parts of the colony, and the male sexual cells later in the older parts.

The male gonophores of *Allopora* resemble those of *Dis-*



In *Pliobothrus symmetricus* the male gonophores are sacs containing a number of small ovoid bodies, which contain spermatozoa, or sperm-cells, in various stages of development. The exact structure of these smaller bodies and their relations to the endoderm were not determined.

Only male specimens of *Stylaster densicaulis* were obtained. Each male ampulla contains two or three ovoid gonophores, which are attached to large offsets of the cœnosarcal meshwork at one end of their longer axes. They have an internal spadix, and in finer structure seem to differ very little from the male gonophores of *Sporadopora*.

Moseley also describes the male gonophores of *Allopورا profunda*, and remarks that they are very similar to those of *Sporadopora*. He does not figure the seminal duct of this genus.

Only one male specimen of *Astylus subviridis* was examined by Moseley. "The male gonophores appear as large rounded lobulated masses resting within the ampullar sacs, and springing from stout offsets of the cœnosarcal meshwork, which pass into the sacs to reach them. . . . The sac as it enlarges becomes gradually pedicellate, and, when mature, is attached to the central mass by a narrow pedicle of some length. The walls of the pedicle are continuous with the ectodermal wall of the sac, which wall contains well-developed nuclei in its substance. Within the sac of the lobule a second sac, composed of a finer membrane, encloses the mature or developing generative elements. The wall of this inner sac is not prolonged into the cavity of the pedicle, but, passing across its commencement, shuts off the main cavity of the lobule from this latter. . . . No rounded spadix, such as that occurring in *Allopورا*, is present in the interior of the lobules." These gonophores seem, from the figures and the description given, to be very similar to those of *Distichopora*.

It is not at all probable that Moseley overlooked the spadix, for in his figure there are represented no fewer than seven gonophores; and he remarks that his material was in a

good state of preservation. The "inner sac" of the gonophore that he mentions and figures is most probably the same as the inner endodermic lining that I have described in both *Allopora* and *Distichopora*. It would be certainly very remarkable if this membrane is not attached to the endoderm of the pedicle in *Astylus*, but this point can only be determined with accuracy by the examination of a continuous series of sections.

The male gonophores of *Cryptohelia pudica* seem to be similar to those of *Astylus*.

### III. THE FEMALE GONOPHORES OF *DISTICHOPORA*.

The position of the female gonophores of *Distichopora* can be readily seen on the female stocks by the prominent swellings on the surface of the corallum. They are usually situated on only one side of the thicker branches, but occasionally there may be found in addition a small cluster on the opposite side.

A section through one of these clusters shows the eggs and embryos in many stages of development, from the minute immature yolkless eggs in the cœnosarcal canals to well-advanced planulæ (fig. 21).

The mature ova (fig. 23, *ovum*) are 0.3 to 0.4 mm. in diameter, and contain a large number of spherical yolk-globules. The large germinal vesicle is situated close to the peripheral border of the egg, and is surrounded by a number of yolk-globules much smaller in size than those of the other part of the egg. The eggs rest in the cup-shaped trophodisc (cf. *Allopora*, Hickson, 7), and is covered by a thin coat of ectoderm and endoderm. The trophodisc is similar to that of the female gonophores of *Allopora*, but not so complicated in its foldings. In transverse section it exhibits twelve pouches at its margin (fig. 24). In vertical section it is simple (fig. 23); the inner and outer pliets that I have described in *Allopora* are not found in this genus.

When fertilisation has taken place the germinal vesicle loses



its sharp outline, and remarkable changes occur in the shape and arrangement of the yolk-globules. My observations are not yet complete of the early stages of the development, but I hope to be able to publish shortly a separate memoir, giving a full account of the development of this form up to the stage when the larva escapes from the ampulla.

During the early stages of development the trophodisc rapidly atrophies, and by the time a layer of columnar epiblast-cells has formed round the embryo no recognisable trace of it can be seen (figs. 22 and 25).

In the meantime young eggs are migrating from the sub-jacent canals to the base of the ampulla, and in many cases before the larva has escaped a new egg, borne by a new trophodisc, occupies a considerable space in the same ampulla (fig. 25).

The young eggs (fig. 22, *ov.*) are frequently seen quite deeply situated in the canal system; those that are nearer to the ampullæ are larger in size and amœboid in shape. As soon as they reach the ampulla they show very minute yolk-granules, which increase in size with the growth of the egg and the development of the trophodisc.

The female gonophores of a few species of Stylasteridæ have already been figured and described by Moseley (10).

In *Pliobothrus symmetricus* "the gonophores are contained in ampullæ which are often sunk deep in the cœnosteum. . . . The ova are solitary, one only being developed in each growing ampulla. Each ovum is developed within the cup of a cup-shaped spadix," i.e. trophodisc. "As the ovum advances in development and increases in size the spadix enlarges with it. Subsequently, however, in later stages, the spadix appears not to increase further, and when in relation with a nearly fully developed planula appears proportionately small."

In *Errina labiata* "the female gonophores are closely similar in structure to those of *Pliobothrus symmetricus*; but there is this great difference, that whilst in *Pliobothrus* the ampullæ and their contained ova and planulæ remain until maturity immersed in the cœnosteum beneath its surface,

in *Errina* the ampullæ project more and more above the surface as development proceeds.

"The spadix in *Errina labiata* is at first cup-shaped, the walls of the cup being composed of a very thick layer of endoderm. The cavity of the cup is directed towards the surface of the coral, and within it rests the single large ovum with its distinct germinal vesicle and spot. Each ampulla contains invariably only one spadix and ovum."

Moseley gives a detailed account of the female gonophore of *Cryptohelia pudica*. In a late stage the trophodisc is "complicated at its margin by subdivision of its lobes, which form a network over one half of the surface of the ovum, terminating in a fringe of numerous tentacula-like lobes."

From these descriptions of Moseley and my own it seems probable that the female gonophores of the various genera of Stylasteridæ are very similar in general structure to one another. Moseley does not describe nor figure an inner endodermal membrane covering the egg, but in other respects his descriptions of the female gonophores of the three genera, *Errina*, *Pliobothrus*, and *Cryptohelia*, agree with mine of *Allopora* and *Distichopora*. The chief point of variation among the different genera is probably the lobulation or branching of the margins of the cup-shaped trophodisc.

I prefer to retain the word trophodisc that I introduced in a former paper to the word spadix used by Moseley for the cup-shaped receptacle of the ovum. This structure cannot be considered to be strictly homologous with the spadix or manubrium of the adelocodonic gonophore of the *Hydromedusæ*. It seems to me to be more probable that it is homologous with the umbrella.

#### IV. THE GONOPHORES OF THE HYDROCORALLINÆ AND HYDROMEDUSÆ COMPARED.

In the absence of a knowledge of the minute anatomy of the gonophores of the *Hydrocorallinæ*, the true position of this group in the classification of the *Hydrozoa* has not yet been very satisfactorily made out.

The peculiar characteristics of the group, namely, the dimorphism of the polyps and the extensive skeleton of carbonate of lime, have not been considered by naturalists to be of sufficient importance by themselves to justify the separation of the Hydrocorallinæ from the Hydromedusæ.

Lankester (9) places them in a separate order of the subclass Hydromedusæ.

In the classification used at Cambridge Balfour placed *Millepora* and the Stylasteridæ in the sub-order Hydroidea of the order Hydromedusæ.

Claus, in his 'Grundzuge der Zoologie,' makes the Hydrocorallinæ the first sub-order of the order Hydromedusæ.

In Jackson's edition of Rolleston's 'Forms of Animal Life' (8) the order Hydroidea is divided into the three sub-orders (1) Tubulariæ, (2) Hydrocorallinæ, and (3) Campanulariæ.

The opinion I have come to, based upon Moseley's researches and my own, is that the Hydrocorallinæ should be placed in an order apart from the Tubulariæ and Campanulariæ (i. e. Hydroidea of Balfour and Jackson).

The classification of the Hydrocorallinæ with the Hydroidea was perfectly justified by the state of knowledge at the time. Both dimorphism and skeletal structures are, comparatively speaking, uncertain features for the purposes of classification, and the character and structure of the polyps and their connecting canal systems show undoubted affinities with many forms of Tubulariæ.

Unless, then, the organs that bear the sexual products can be shown to differ very widely from those of the Hydroidea, and present characteristics peculiarly their own, the Hydrocorallinæ must remain in the position that is assigned to them by some authorities in the order Hydroidea.

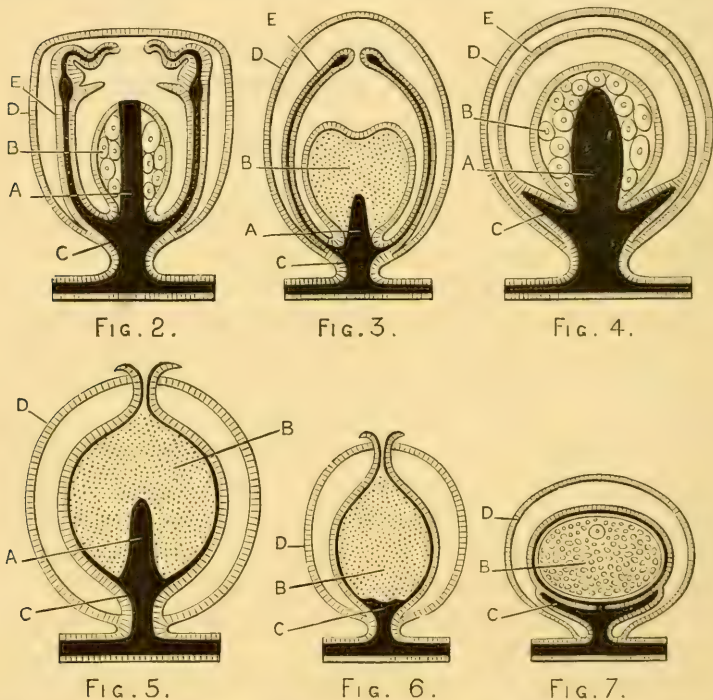
These considerations demand a careful and exhaustive comparison of the typical gonophores of the Tubulariæ, and of those Hydrocorallines that are at present known to us.

To aid in the discussion of the homologies I have given on p. 390 diagrammatic figures representing the structure of (Woodcut 2) a phanerocodonic medusa, (3) a medusa of *Mille-*



pora, (4) an adelocodonic medusa, (5) the male gonophore of *Allopora*, (6) the male gonophore of *Distichopora*, (7) the female gonophore of *Distichopora*.

Figs. 2 and 4 are copied from Allman with this modification, that both endodermal tissue and endodermal cavity are



WOODCUTS 2—7.—The structure of the different gonophores compared. Diagrammatic sections of—2. A phanerocodonic gonophore. 3. The medusa of *Millepora*. 4. An adelocodonic gonophore. 5. Male gonophore of *Allopora*. 6. Male gonophore of *Distichopora*. 7. Female gonophore of *Distichopora*. A. Manubrium. B. Gonad. C. Endoderm. D. Ectoderm. E. Umbrella.

represented in black. The diagrams are modified in this way, because no important morphological distinctions can be drawn between endodermal structures possessing a cavity and those that do not. For example, no one would think of drawing a



fine morphological distinction between the dactylozooids of *Millepora* and those of *Allopora* because in the case of the former there is a lumen and in the case of the latter the endoderm is solid.

In comparing the structure of the phanerocodonic medusa and the medusa of *Millepora* a very general similarity may be observed.

In both there is a centrally placed manubrium (A).

In both the generative elements (B) are developed between the ectoderm and endoderm of the manubrium.

In both there is a contractile bell umbrella, from the centre of whose concavity the manubrium is suspended; in both this umbrella is composed of a centrally placed sheath of endoderm covered by a sheath of ectoderm on both sides; and in both the gonophore lies in a gonangial cavity of ectoderm, which, before the medusa is set free, is continuous with the ectoderm of the outer wall of the umbrella.

The principal points in which these two forms differ from one another are these:

The manubrium of 2 possesses a mouth.

The manubrium of 3 does not.

There is a system of canals (longitudinal and ring) in the umbrella of 2.

There are no canals in the umbrella of 3.

There are tentacles and sensory epithelium at the margin of the umbrella in 2.

There are no tentacles or sensory epithelium at the margin of the umbrella of 3.

There is a velum in 2.

There is no velum in 3.

Too much stress should not be laid upon any of these points of difference, for it is quite possible that tentacles, eyes, or auditory organs, a velum and a system of gastro-vascular canals, may be subsequently developed in the medusa of *Millepora* after it is set free.

It is of importance to note, however, that these organs are not developed while the medusa is still attached to the parent

stock, as they are in the typical phanerocodonic medusa of the Tubulariæ.

Comparing the medusa of *Millepora* with the adelocodonic gonophore (fig. 4) of *Hydromedusæ*, the following points of difference may be observed :

There is a codonostome in the former, there is none in the latter.

In the former the endoderm extends almost to the margin of the umbrella, in the latter the endoderm is reduced to a shallow cup surrounding the base of the manubrium.

In other respects the two gonophores are practically similar.

Comparing the adelocodonic gonophore (fig. 4) with the male gonophore of *Allopora* (fig. 5), two points of difference may be observed. In the first place the endoderm completely surrounds the gonad in the latter, excepting at a small aperture at the distal pole, where it forms the inner wall of a narrow seminal duct. Secondly, there is no layer of ectoderm between this endoderm and the gonad in *Distichopora*. In the adelocodonic gonophore there are two layers of ectoderm between the gonad and the wall of the gonangium.

The male gonophore of *Distichopora* (fig. 6) resembles that of *Allopora* (fig. 5) in all respects except one, namely, that in the former there is no manubrium.

The female gonophores of the two genera of *Stylasteridæ* resemble the male gonophores in most respects, but in the former there is a more complicated plicating of the base to form a nourishing disc (trophodisc), and no structure corresponding to a manubrium can be observed.

Do these gonophores of the *Hydrocorallinæ* represent stages in the degeneration, or do they represent stages in the evolution of the free medusiform gonophore?

It would be more satisfactory, perhaps, to leave these questions to be answered at a time when we are better acquainted with the minute anatomy of the gonophores of other species of *Millepora* and the other genera of the *Stylasteridæ*.

The very convincing proofs that have been brought forward by Balfour, Weismann, and others, showing that the gonophores

of the Hydroidea, however simple in structure, represent stages in the degeneration of medusæ, may lead to the conclusion that these gonophores of the Hydrocorallines are also degenerate medusæ; and it is necessary to issue a warning that this is probably not the case.

That the medusa of *Millepora* is not degenerate but primitive in its simplicity must be apparent.

In the course of its development there is no abbreviation nor any trace of organs that were at one time functional and have since become rudimentary. Moreover, it cannot be considered at all probable that a free-swimming medusa, bearing immature spermatozoa, would have lost its mouth, tentacles, sensory organs, endoderm canals, and velum; or, if it is a degenerate medusa, that the development of these organs would be postponed until after its escape.

The only view that seems to me to be at all tenable is the one that considers the medusa of *Millepora* to be primitive in its simplicity.

As regards the male gonophores of *Allopora* and *Distichopora*, there is without doubt a close similarity in appearance between certain stages in the development of the male gonophores of both these genera and the younger stages of the medusæ of such forms as *Pennaria* and other Tubularians (cf. this paper, Pl. XXX, and Weismann (12), pl. xvii, fig. 3); and the manubrium of *Allopora* is undoubtedly closely similar in general appearance to the manubrium of the adelocodonic gonophore of many of the Tubulariæ. In fact, the gonophores of some of the Hydroidea, such as *Clava* (Allman) and *Corydendrium* (Weismann), are much less like adelocodonic medusæ, even when they reach their full development, than are these gonophores of *Allopora* and *Distichopora*.

If it could be shown that the inner membrane covering the spermarium is derived from the ectoderm and is not endodermic as I have described it, and that structures corresponding to the "glockenkern" do occur in the development of these gonophores, then my principal objections to the view that they are degenerate medusæ fall to the ground. A very

careful examination of my sections of gonophores in all stages of development convinces me that there is in these forms no true "glockenkern," and that the two membranes covering the gonad are truly homologous with the two membranes covering the ova, namely, an outer ectodermic membrane and an inner endodermic membrane.

The manubrium of the gonophore of *Allopora* is, I believe, strictly homologous with the manubrium of the medusa of *Millepora*; that is to say, it is a subsequent endodermal ingrowth into the spermarium developed for the purpose of affording increased nourishment to the rapidly increasing spermoblasts.

These gonophores, then, do not represent, in my opinion, stages in the degeneration of medusæ. The *Stylasteridæ* never possessed free-swimming medusæ, I believe, although their gonophores may indicate to us some of the stages that the medusæ of *Hydroidea* passed through in the course of their phylogeny.

Before entering into a discussion of the meaning of the gonophores of the *Hydrocorallines*, it is necessary to consider briefly the principal views that have been put forward concerning the primitive or ancestral form of the *Hydrozoan*. Is it probable from the evidence at our command that the ancestral form was a fixed colonial hydroid, or was it like a scyphistoma larva (*Hydra tuba*); or, lastly, was it a floating *Hydra* or *actinula*?

Balfour says, "A condition like that of *Hydra*, in which the ovum directly gives rise to a form like its parent, is no doubt the primitive one, though it is not so certain that *Hydra* itself is a primitive form. The relation of *Hydra* to the *Tubulariæ* and *Campanulariæ* may best be conceived by supposing that in *Hydra* most ordinary buds did not become detached, so that a compound *Hydra* became formed; but that at certain periods particular buds retained their primitive capacity of becoming detached, and subsequently developed generative organs, while the ordinary buds lost their generative function."



Weismann's view is similar to that of Balfour. He says, "Die niedrigste d. h. einfachste Form der heute lebenden Hydroiden ist wohl Hydra; es scheint mir wenigstens für jetzt kein Grund vorzuliegen, sie für eine rückgebildete Form, wohl aber manche Gründe sie für eine sehr alte Form zu halten, wie oben schon genauer begründet wurde, und wie es auch so von den meisten Forschern angenommen wird" (12).

Both of these authors considered that the primitive type of Hydrozoan was a simple sessile form more or less similar to our modern Hydra, and that the medusa originated by the modification of individuals bearing the sexual cells that were budded from, and set free from, the primitive simple sessile Hydra.

Lankester says, "The particular form which the proximate ancestor of the Hydrozoa took is most nearly exhibited at the present day in *Lucernaria*, and in the *scyphistoma* larva (*Hydra tuba*) of *Discomedusæ*. It was a hemispherical cup-like polype with tentacles in multiples of four, with four lobes to the wide enteric chamber. This polype, after passing a portion of its life fixed by the aboral pole, loosened itself and swam freely by the contractions of the circular muscular fibres of the hypostome (sub-umbrella), and developed its ovaria and spermata on the inner walls of the enteric chamber. This ancestor possessed, like its descendants, a very marked power of multiplication, either by buds or by detached fragments of its body. Accordingly it acquired definitely the character of multiplying by bud formation during the earlier period of its life; each of the buds so formed completed in the course of time its growth into a free-swimming person. We must suppose that the peculiarities of the two phases of development became more and more distinctly developed, the earlier budding phase exhibiting a more elongated form and simple enteric cavity (*Hydra* form), which subsequently became changed in the course of ontogeny into the umbrella or disc-like form, with the coalesced enteric walls and radial and circular surviving spaces (*medusa* form). And now the ancestry took two distinct lines, which have given rise respectively to

the two great groups into which the Hydrozoa are divided—the Scyphomedusæ and the Hydromedusæ.”

Another view has been put forward by Brooks (3), who, from a consideration of the developments of the Trachomedusæ and Narcomedusæ, comes to the conclusion that the ancestral form was a simple solitary floating or swimming Hydra.

It does not seem to me to be at all clear that Claus previously expressed the same view in the ‘Grundzüge der Zoologie,’ for although he says that Hydra is certainly not a primitive form, that the medusa is a higher form than the polype, and that intermediate forms between the medusa and polype are represented by the actinula of Tubularia and Tetrapteron volitans, he does not commit himself to the view that the ancestral Hydrozoan was a free-swimming Hydra-like larva.

Böhm (2), on the other hand, expresses his views very clearly: “Eine der nächsten Nachkommen der uralten Gastraea muss als die Stammform der Zoophyten, eine nicht weit von ihr entfernte als die der Hydromedusen angesehen werden. Bei der hypothetischen Construction der letzteren hat man zwischen drei Möglichkeiten zu wählen.

“Entweder war diese schon entschieden polypoid ihre nächsten Nachkommen waren Polypen, und die Medusen haben sich erst später aus diesen entwickelt.

“Oder sie war ganz medusoid, die Medusen die primären die Polypen die secundären Nachkommen.

“Oder schliesslich es war eine intermediäre zwischen Polypen und Medusen stehende Form, und Polypen wie Medusen haben sich von ihr aus nach zwei verschiedenen Richtungen hin entwickelt.

“Die letztere Annahme schient mir manche Gründe für sich zu haben. Denn der lange Weg vom wenig differentzierten feststehenden Polypen bis zur hochausgebildeten freischwimmenden Meduse wird wesentlich abgekürzt durch die Annahme einer Mettelform.”

Notwithstanding the arguments of these authors, it is not easy to believe that the free-swimming actinula represents an

ancestral type of Hydromedusan. The parasitic or semi-parasitic habits of the actinula of most of the Narcomedusæ suggest that it is an extremely modified form, and it seems to me to be extremely hazardous on the part of Brooks to base his phylogenetic considerations upon such a weak and slender foundation. The views of the earlier writers that the sessile form is the more primitive, that in those cases in which the medusa develops directly from the egg the trophosome has disappeared from the developmental cycle, seem to be more probable.

It is not necessary to enter further into the discussion of these extreme speculative questions.

I have referred to them not in the hope of adding anything new, nor of throwing light upon them, but in order that I may place clearly before the reader the position I take with regard to them.

It seems to me to be more satisfactory to regard the sessile trophosome rather than the free-swimming actinula as the primitive type, and the medusa as a structure produced originally by a polypoid colony for the nourishment and distribution of the gonads.

Having thus stated my opinion as to the original form of Hydroid, it is necessary to go further and express an opinion as to the mode in which medusæ originated.

The views of Weismann and Balfour on this question are as nearly as possible identical. They supposed that the medusa originated by certain buds bearing the primitive sexual cells, retaining their primitive capacity of being detached from the parent, and that such buds became modified for a free-swimming existence. According to these views the medusa is homologous with a polype, it is simply a modified trophosome, or that trophosomes and gonophores are both modifications of some common type.

Huxley's original view that the gonophore is a peculiar sexual organ has in recent years been subject to a storm of criticism, and there are very few naturalists of the present day who would defend the position he took. "A medusoid, though

it feeds and maintains itself, is in a morphological sense simply the detached generative organ of the hydrosoma on which it is developed."

The gonophores of the Hydrocorallinæ do not seem at first sight to throw much light upon these questions. If we arbitrarily assume that they are degenerate medusæ comparable to the adelocodonic gonophores of the Tubulariæ and Campanulariæ, we cannot expect to find in them any evidence to support either the one view or the other. But there is no reason to suppose that they are degenerate medusiform gonophores. Neither in *Millepora*, nor in *Allopora* and *Distichopora*, are there any features in development that suggest rudimentary structures of medusæ.

If they are not degenerate structures, then, but gonophores of a primitive type, how can we reconcile the medusa of *Millepora*, which is a metamorphosed polype, with the gonophores of *Allopora* and *Distichopora*, which show no trace of polypoid or medusoid structure?

The explanation I would suggest is briefly as follows: When the ova or sperm-mother cells reach a certain size and are too large to move freely in the canal system, they set up a local stimulus or irritation, which causes a cup-shaped folding of the adjacent canal or polype wall. This cup-shaped fold being of advantage to the sexual cells during their maturation, by affording increased facilities for nourishment and by increasing the size of the cavity by solution of its walls, has been modified into a definite form in each species by natural selection. When the sexual cells arrive at their maturity the nourishment afforded by these cells is no longer necessary, and consequently the stalk of connection with the canals becomes constricted until the gonophore is set free in the cavity of the ampulla. In the ancestral form of the *Millepora* a ready access to the exterior was open to the separated gonophore by way of the dactylopore, and thus the detached gonophore was able to escape and lead a free-swimming existence.

It is reasonable to suppose that all the cells of the colony of a *Millepora* are capable of a certain amount of contractility,



and that the slight power of contractile movement that the original free gonophore possessed being of advantage to the species—by enabling the gonophore to keep afloat longer and thus spread the sexual products farther—was increased by natural selection. Similarly the rim of the gonophore cup was produced until it assumed the size and shape of a medusa.

The whole of this hypothesis of the origin of the medusæ rests upon the supposition that the sexual cells when they reach a certain size set up a local irritation or stimulus, causing a cup-shaped growth of the cœnosarc in its immediate neighbourhood.

Is it reasonable to suppose, in the first place, that the gonads when they reach a certain size do produce a local stimulus or irritation? In young immature stocks there is no trace of ampullæ or other receptacles in the cœnosteum of sufficient capacity for the mature gonads. Nor are there found in stocks that are bearing but few sexual organs any empty cavities in the cœnosteum. It is almost certain, then, that the gonads, when they reach a certain size, cause a stimulus to certain cells to secrete an acid (?) which dissolves the lime of the cœnosteum and causes an ampulla to be formed. There can be no doubt, then, that the sexual cells do cause one kind of stimulus to the tissues.

But is a local irritation or stimulus likely to cause any such modification as circumferential folding of the canals in its neighbourhood?

The only direction in which we can look for an answer to this question is to the effects caused by the irritation of foreign substances and parasites. The Hydrocorallines, like most of the corals, are subject to the attacks of many kinds of parasites. Worms, molluscs, barnacles, and other forms may be seen in every specimen that is examined.

When the colony is attacked by such a form as *Tetracilita*, for example, the cœnosarc at the immediate spot on which the parasite settles is killed, but this does not cause an atrophy of the surrounding canal system. On the contrary, a pronounced hypertrophy of the canal system immediately surrounding the

parasite takes place, and in time it grows round and over the parasite until it is almost buried in its substance. An examination of other forms of coral will show similar examples of parasites and other foreign bodies covered by an hypertrophied growth of the *cœnosarc*.

The formation of the corbula of *Aglaophenia* may be accounted for by a similar explanation. The stimulus of the growing blastostyles causes, not only an increased activity in the growth of the lateral branchlets, but a growth in such a manner as to enclose the blastostyles in a cup.

Similarly the various kinds of animal galls found in Hydroids and Alcyonarians are probably caused by a circumferential hypertrophy of the tissues surrounding the parasitic pycnogonid, crab, or mollusc.

From this evidence, then, it does seem probable that a local stimulus or local irritation of the *cœnosarc* of these forms causes a growth of the tissues which gradually folds over the seat of the irritation.

If this is the case, then, the production of a very rudimentary and imperfect umbrella-shaped structure is a physiological result of the stimulus caused by the growth of the sexual cells, and the medusa is simply a modification, produced by natural selection, of such a structure.

If this view is a reasonable one, we get over the principal difficulty in accepting the view that the ancestral Hydrozoan was a colonial *Hydra* form.

One of the chief features of the higher Protozoa and of the *Cœlenterata* is the power they possess of forming large colonial organizations by asexual reproduction. And it is reasonable to suppose that when the primitive Hydrozoan became differentiated off from its colonial Protozoan ancestry it retained the power of forming colonies by fission or gemmation.

It has seemed to me improbable that *Hydra* can be closely related to the ancestral type, because it does not possess this power.

If this view of the origin of medusæ is correct, there is

no difficulty in believing that the ancestral form was a colonial trophosome, and that medusæ of different kinds may have originated quite independently of one another from the Hydroid stocks.

The original position of the gonads was the centre of the concavity of the umbrella. As they became larger and larger in phylogeny a conical growth of the endoderm, with respiratory and nutritive functions, penetrated them, and became the manubrium. All of these stages may be seen repeated in the ontogeny of the medusa of *Millepora*. When a mouth was formed at the end of the manubrium the gonads were in some forms (anthomedusæ) restricted to the sides of that organ; but in other forms (leptomedusæ) they were shifted to a more convenient place in the radial canals. According to my view, then, the manubrium of the male gonophore of *Allopora* does not prove that it is a degenerate medusa, but, rather, that it is one stage further than *Distichopora* on the road that all medusæ have travelled in the early history of their phylogeny; that is to say, a stage with a larger spermarium, and a special process of endoderm for its more perfect nourishment and respiration.

Another question arises in connection with the gonophores of the *Hydrocorallinæ* that at one time would have been considered one of vital importance.

In the description given above of the development of the medusa of *Millepora*, I have shown that it is formed by a metamorphosis of a dactylozoid. This would support the view, then, that the medusa is a modified trophosome.

In the description of the development of the gonophores of *Allopora* and *Distichopora* I do not mention the zooids at all. The gonophores are not developed in these genera (figs. 12, 19) in connection with either the gastrozooids or dactylozooids, they arise quite independently from the cœnosarcal canals. They have no particular relation to the systems in which the zooids are arranged, and there is every reason to suppose that they are quite independent of them. Further, these gonophores are not, according to my view, degenerate

medusæ. They must, therefore, be special organs of the colony bearing the gonads.

To those naturalists who believe that there is a sharp distinction to be drawn between the idea of the "individual" and the "organ" in the animal kingdom, these apparently contradictory cases must be very puzzling. In the one case they would say the gonophore is an "individual;" in the other, it is an "organ."

I am not inclined, however, to believe that it is possible to draw a sharp distinction between these two ideas. They are relative ideas, as Claus (5) maintains, just as "cell" and "tissue," "individual" and "colony," must be.

The stimulus of the sexual cells of a certain size would produce the same effect if they were formed in the cœnosarcal canals or the zooids; but natural selection has stepped in in the case of the Hydrocorallines, so that in the case of *Millepora* the gonads do not produce this effect until they reach the zooids, and, in the case of the Stylasteridæ, not until they reach certain parts of the canal system.

The two kinds of gonophores are, then, to my ideas really homologous, although in the one case they have reached such a stage of development as to justify us in considering them "individuals," while in the other case they cannot be considered more than sexual "organs."

### General Conclusions.

1. In *Millepora murrayi* (*sp. ?*) the male gonads are borne by medusæ which escape from the ampullæ in which they are developed before the spermatozoa are matured.

2. The ova of this species are, like the ova of *Millepora plicata*, extremely small and alcithal. They move in an amœboid manner in the cœnosarcal canals, and do not ultimately rest in gonophores, nor in any specialized portion of the system.

3. The medusæ of *Millepora murrayi* have no radial nor ring canals in the endoderm of the umbrella, no velum, no sensory organs, and no mouth.



4. The medusæ are formed by a metamorphosis of an ordinary zooid; in the majority of cases dactylozooids, but in others gastrozooids.

5. The sperm-cells originate in the ectoderm of the cœnosarc and wander into the ectoderm of the zooids, where they fuse into aggregations to form a spermarium.

6. The young spermarium is formed at the distal extremity of the dactylozooid, and when it has reached a certain size it causes a retrograde metamorphosis of the tissues. The tentacles flatten out and disappear, and the zooid loses all its characteristic features.

7. A cup-shaped outgrowth next appears which forms the umbrella of the medusa, and subsequently a conical growth of the endoderm penetrates into the substance of the spermarium and forms the manubrium.

8. The male gonophores of *Distichopora* occur in groups of two or three in each ampulla in different stages of development. The gonad is supported by a small cup-shaped trophodisc, and enclosed in a double sac of ectoderm and endoderm. At the distal pole of the ripe gonophore there is a short seminal duct.

9. The male gonophore of *Allopora* differs from that of *Distichopora*, in the fact that it is provided with a club-shaped endodermal manubrium or spadix.

10. The female gonophore of *Distichopora* resembles that of *Allopora* described in a previous paper; but the folds of the trophodisc are not so complicated.

11. The gonophores of the *Hydrocorallinæ* are not degenerate medusæ.

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## DESCRIPTION OF PLATES XXIX &amp; XXX,

Illustrating Mr. Sydney J. Hickson's paper "The Medusæ of *Millepora murrayi* and the Gonophores of *Allopora* and *Distichopora*."

*Calc.* The calcareous skeleton or cœnosteum. *Cœn.* The cœnosarcal canals. In the superficial regions the canals are crowded with zooxanthellæ. *Ect.* Ectoderm, coloured red. *End.* Endoderm, coloured blue. *Gon.* The ectodermal lining of the ampulla forming the wall of the gonangium. *Man.* Manubrium of the medusa. *Nemat.* Large nematocysts guarding the dactylopores. *op.* Operculum of modified ectoderm cells covering the pore of the ampulla. *Sperm.* Spermarium. *Sperm. S<sub>1</sub>.* Spermospheres or aggregations of spermospheres in the ectoderm of the zooids. *Sperm. S<sub>2</sub>.* Young spermospheres in the ectoderm of the canals. *Tent.* Retracted tentacles. *Umb.* Umbrella of the medusa, consisting of a solid endoderm covered on both sides by ectoderm.

## PLATE XXIX.

*Millepora murrayi*.

FIG. 1.—Section through a retracted dactylozoid of *Millepora murrayi*, showing a number of spermospheres (*Sperm. S<sub>2</sub>.*) in the ectoderm of the cœnosarc, and in the ectoderm (*Sperm. S<sub>1</sub>.*) at the base of the dactylozoid.

FIG. 2.—Section through a retracted dactylozoid, showing a single small aggregation of spermospheres (*Sperm. S<sub>1</sub>.*) in the ectoderm at the base of the dactylozoid.

FIG. 3.—Section through a retracted gastrozoid, showing an aggregation of spermospheres in the ectoderm. The gastrozoids may be readily distinguished from the dactylozoids by the presence of a mouth and by the large endoderm cells, the peripheral portions of which are filled with mucus. Just below the gastrozoids may be seen a plate of vacuolated ectoderm cells in section, which forms the last tabula of the gastropore.

FIG. 4.—Section through a dactylozoid, showing a large aggregation of spermospheres on its side in a condition very similar to that I have described in *Millepora plicata* (6). The spermospheres have caused a very considerable depression in the dactylozoid, and are partially covered by the surrounding parts.

FIG. 5.—An aggregation of spermospheres at the peripheral extremity of a dactylozoid. The tentacles (*tent.*) are visible.

FIG. 6.—An aggregation of spermospheres (*Sperm. S<sub>1</sub>.*) at the peripheral

extremity of a dactylozoid, sunk in a cup-shaped receptacle. At *Umb.* may be seen the first trace of the formation of the umbrella by the growth of the endoderm. The position of the tentacles is still indicated by the rows of small nematocysts.

FIG. 7.—Section through another dactylozoid, showing a still further growth of the folds forming the umbrella. All trace of the tentacles has disappeared.

FIG. 8.—Section through a young medusa of *Millepora*. The form of the dactylozoid is completely lost. The endoderm of the umbrella is solid, and much thicker than it is in later stages. The opening of the dactylopore can still be traced, although it is blocked with the thickened ectoderm cells. The pore is guarded by nematocysts (*Nemat.*).

FIG. 9.—Section through another medusa. The umbrella is not completely developed, but the endoderm is much thinner than it is in Fig. 8. The spermarium is much larger, but there is no trace of a manubrium. The dactylopore is completely closed by an operculum (*op.*) formed by flattened strap-shaped ectoderm cells.

FIG. 10.—Section through another medusa, with a well-developed manubrium (*man.*), containing a cavity continuous with a large canal. The umbrella walls are much thinner than they are in the specimens drawn in Figs. 8 and 9, except at the margin.

FIG. 11.—Section through a medusa that lies freely in the gonangium. It is not connected organically with the colony at any point. It is probably ready to escape. The umbrella (*Umb.*) is extremely thin, except at the margins. There is a small cavity in the endoderm, but there is no mouth. There are no tentacles, velum, nor sensory bodies on the margin of the umbrella. Between the codonostome and the superficial ectoderm there is a layer of mucus.

## PLATE XXX.

FIG. 12.—Transverse section through a decalcified branch of *Distichopora*, showing the male gonophores lying in the ampullæ. One, two, or three gonophores occur in each ampulla. At the edges of the branch are situated the rows of dactylozooids (*Dact. Z.*) and gastrozooids (*Gast. Z.*).

FIG. 13.—Section through an ampulla of *Distichopora*, containing two young male gonophores. Each of these is supported by its own trophodisc containing a large lumen.

FIG. 14.—Section through an ampulla of *Distichopora*, containing three male gonophores in different stages of development. The largest of these (1) contains ripe spermatozoa, and shows on its distal pole a conical cap of



cells, the undeveloped seminal duct. The trophodisc (*troph.*) is reduced to an irregular mass of endoderm cells.

FIG. 15.—Section through a very young male gonophore of *Distichopora*. The young spermarium (*sperm.*) lies apparently between the ectoderm and endoderm of the bud, but the endoderm is cup-shaped, and the margins of the cup project between the ectoderm and the proximal hemisphere of the spermarium.

FIG. 16.—Section through an older male gonophore of *Distichopora*, showing the spermarium covered by the two membranes, a thin nucleated ectoderm and a thinner non-nucleated endoderm, which is continuous with the endoderm of the trophodisc.

FIG. 17.—Section through the earliest stage I have found of the formation of the seminal duct. The ectodermic and endodermic elements are from the very first quite distinct from one another.

FIG. 18.—Section through a seminal duct of a ripe male gonophore, open to the exterior.

FIG. 19.—Section through a portion of a decalcified branch of *Allopora*, showing three male gonophores lying in their ampullæ. As a rule, only one gonophore is found in each ampulla; but one case is figured (gonophore 2) in which a large gonophore and a very young bud occur in the same ampulla.

FIG. 20.—Section through a nearly ripe male gonophore of *Allopora*, showing the club-shaped endodermal spadix, and the two membranes (*Ect.* and *End.*) surrounding the spermarium.

FIG. 21.—Section through a portion of a decalcified branch of a female stock of *Distichopora*, showing a number of ova and planulæ in various stages of development lying in their ampullæ.

FIG. 22.—A portion of the same as Fig. 21, more highly magnified. The ampullæ are occupied by planulæ. Below the ampullæ there may be seen in the endoderm of the canals some very young eggs, containing no yolk-granules and showing blunt amœboid processes.

FIG. 23.—An ovum of *Distichopora* that is nearly mature, as seen in section. The germinal vesicle (*Germ. Ves.*) lies near the superficial side of the egg, and is surrounded by small yolk-granules. The trophodisc is simple, in vertical section, and contains a pronounced lumen.

FIG. 24.—Transverse section through an ovum and trophodisc of *Distichopora* in the plane represented by the line  $x x'$  in Fig. 23, showing the twelve pouches of its margin.

FIG. 25.—Section through an ampulla of *Distichopora*, containing a planula, and below it a young ovum in a young trophodisc.



On a Red Pigment-forming Organism,  
*B. corallinus* (?).

By

**Charles Slater, M.B.(Cantab.).**

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With Plate XXXI.

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THE number of micro-organisms known which form red pigments is already considerable, and include the *M. prodigiosus*, *B. indicus*, *B. ruber*, and *B. rouge de Kiel*. The organism described below differs from any of these in morphology, cultural characteristics, and tint of pigment produced. It occurred as a coral-red, slow-growing, circular, non-liquefactive colony on a gelatine plate which had been used in an examination of the tap water of the laboratory. As the plate had been withdrawn from the moist chambers and examined at least once before the colony appeared, it is impossible to say whether it was derived from the water or was an air contamination. It was most probably the latter.

The colony was found to consist of short, thick bacilli with very rounded ends. Their breadth, which is very constant, is about  $1\ \mu$ , and the average length of the individual cells from 2 to  $3\ \mu$ . Very frequently two cells are joined end to end, but it is unusual for more than two fully formed cells to remain in apposition. The organism is motile, the movements being of a rolling recurving character, with but slow motion of translation, except in the case of certain apparently young short cells. The character of the motion recalls that of *B. megaterium*, but is somewhat more active. The apparent curving and

straightening movements seem to be due to the rolling motion of a slightly curved organism round its longitudinal axis.

By far the most noticeable characteristic is the highly refringent nature of the poles of the cells. This refringence is noticed in a very large proportion of the cells of a culture examined at any stage of growth, and will be again referred to.

On gelatine peptone growth takes place easily, though not very rapidly, and does not produce liquefaction of the medium. The growth forms a regular, raised, moist-looking, somewhat glistening streak on the surface of the gelatine, and is frequently surrounded by a white opalescence due to a deposit of oxalate of calcium. The colour is pinkish or coral red, and though this tint deepens during the first week the coloured colonies are not preceded by any definite uncoloured stage, as is the case in the *Prodigiosus*, &c.

In a stab culture the growth takes place very scantily along the needle track, and remains colourless. On the upper surface the growth spreads from the point of inoculation, and colours well. The organism is distinctly aërobic.

On agar the growth is a little slower, and is rather more vermilion in colour. The addition of carbohydrates to the agar seems to make no difference in either the rapidity or colour of the growth. In the case of the *B. rouge de Kiel*, M. Laurent (*Ann. Inst. Past.*, iv, 465) has shown that the colour is not produced in the presence of carbohydrates. The addition of glycerine to the medium is, however, inhibitory to the growth of the bacillus now described. At the most there is a slight pink growth at the margin of the fluid which always collects at the bottom of these tubes.

In liquid media the growth is never copious, and the colour extremely ill-developed. The media tried were ordinary bouillon, bouillon with carbohydrates, and Pasteur's fluid with and without sugar. The addition of sugar both to the bouillon and the Pasteur's fluid certainly increases the growth and assists the colour formation. The growth in these media tends to collect at the edge of the fluid and to attach itself to



any extraneous body, and forms delicate, slightly resistant, gelatinous films. The organism in these films does not appear to have any distinct morphological peculiarities. There is a decided tendency to the formation of a gelatinous material round the cells in the cultures on solid matter, especially on potato.

On potato the growth is copious and the colour well developed. The organism forms a raised irregular waxy-looking coating with a bright reflecting surface. The growth is gelatinous, adherent to the surface of the potato, and the colour is much the strongest in the superficial layers. A greyish blue discoloration of the potato occurs round the growth, appearing early but subsequent to the development of the red pigment, and obviously connected with the growth of the organism. The potato acquires within the zone of grey pigment a slight alkaline reaction, but no distinct odour is developed, as in the case of *M. prodigiosus*. In old potato tube cultures the pigment becomes a pale chocolate-brown.

**Temperature.**—The organism grows well at the ordinary temperature, and has its optimum between  $20^{\circ}$  and  $23^{\circ}$ . Above this the growth is slower, and at  $37^{\circ}$  ceases, but the cultures grow rapidly again on removal to a lower temperature. Exposure for one hour to a temperature of  $60^{\circ}$  kills the organism. Simple drying at  $37^{\circ}$  on a cover-glass does not impair the vitality.

**Pigment.**—The pigment in this organism is largely contained in the cells, and is not an excretion. It cannot be extracted by simple shaking with water, ether, alcohol, or chloroform, but requires to be first liberated by disruption of the cells by boiling. It is set free by boiling in water, but is not dissolved. Alcohol and chloroform dissolve it easily, but ether does not. It has not been obtained in a crystalline form. When acted on by alkalis it is turned a yellowish brown, and this is probably the reason of the change of colour in old potato cultures. Acids restore the colour affected by alkalis, but do not themselves cause any change in the original pigment. With the spectroscope no absorption bands could be detected,

the reddish solution simply cutting off the blue end of the spectrum.

The production of the pigment is unaffected by light, taking place as well in the dark as in diffused light.

The pigment-producing power is remarkably constant, and in no case have any colourless colonies been obtained when free access of air is possible. In stab cultures, and in the depths of liquid media, the colour is absent.

If recent cultures of the organism on gelatine or potato are examined, the growth is found to consist chiefly of cells scarcely twice as long as broad, and so rounded at the ends as to appear oval. These small cells are actively motile, and the protoplasm appears to be collected as a refringent mass at each pole. These cells are not unfrequently united in pairs, connected by a thin filament. The bicellular organisms are actively motile, and present the appearance of a flagellated organism. They are especially noticeable in bouillon cultures. Besides those two forms which seem to be characteristic of young and active growth, there occur cells which are three to four times as long as broad, some showing signs of division into the bicellular stage, and others no trace of this separation.

The organism increases by fission, which is somewhat slow, a cell dividing at the temperature of 15° C. in about twelve hours. The most noticeable feature in this organism at whatever stage examined is, as mentioned above, the irregular refractive power of its protoplasm, and its tendency to collect in masses, having various positions in the organism, but very frequently to be found at the poles of the cells. Stained specimens present, also, a very great variety of appearances, owing to their extremely irregular staining. A stained preparation of this bacillus recalls, very strongly, a culture of the *Bacillus typhosus* when in the condition which shows the "clear space" and the so-called spores, and the appearances presented by this pigment-forming organism are not without interest when compared with those of the pathogenic microbe. Certain cells show, both when stained and unstained, caps of

protoplasm, with an intermediate hyaline scarcely stained cell body. These resemble the clear space cells of the *B. typhosus*. In other cells the refractive mass is gathered into a distinct rounded spore-like mass at each pole, and in stained preparations a very common appearance is that represented in fig. 3, where a strongly stained central mass is flanked by two oval unstained bodies, apparently corresponding to the refringent masses, and having the appearance of terminal spores.

Büchner has shown that the refringent terminal mass, described as a spore in the case of *B. typhosus*, was really a collection of protoplasm, and stained deeply, and did not correspond to the unstained oval space in stained specimens. Similarly, by direct staining on the slide, it may be seen that the rounded refractive polar masses are protoplasmic and stain deeply, and that the two forms *a* and *b* in figs. 1 and 2 are not corresponding stained and unstained appearances, but the forms *a* and *c*. That the oval unstained spaces seen in fig. 3 are not spores is indicated by their rather irregular shapes and somewhat faint outline, which suggest rather the space left by the withdrawal of the central protoplasm from that part of the cell. Further, no attempts at differential staining or any of the usual methods of spore staining have been successful, and no free spores can be demonstrated in the cultures. The cultures containing these forms are sterilised by one hour's heating to 60° C.

Regarding the irregularity of staining as due to irregularities in the distribution of the cell contents, the question arises as to whether the forms noticed are produced artificially in preparation, or are the expression of normal changes in the cells, or are the results of degenerative processes. That the various forms are not produced artificially is shown by the fact that similar results are obtained whether the specimens are fixed by heat, alcohol, or simply drying, and that a parallel irregularity is seen in fresh specimens, and in preparations made by staining directly on the slide without fixation.



Bütschli has advanced the theory that the cell contents of bacteria represent the nuclei of other cells, while the protoplasm is reduced to an extremely thin layer, in many cases only represented by the cell wall. He bases his views ('Bütschli ueber den Bau der Bacterien, etc.,' Leipzig, 1890) on the study of the large flagellated organisms found in sulphur waters, which he shows to possess an internal cell substance, having the structure and staining properties of nuclei, and containing the granules of Ehrlich. The refractive portions of the organism described in this paper are strongly stained by the ordinary nuclear stains, such as alkaline aniline dyes, and especially logwood. On this view the forms which have been described as occurring in young freely growing cultures, viz. the short oval cells with refractive poles occurring either singly or in pairs, would apparently be the forms resulting from direct division, and the polar collections of protoplasm would represent the direct division of the nucleus. The successive stages of this division would be represented (fig. 4, *a*) by—1, the cell, with refringent poles; 2, a form resembling a large diplococcus, stained throughout, and formed by division of stage 1—this form occurs but rarely; 3, a bicellular organism found by the growth of stage 2. In this third stage the cell stains at first equally throughout, but soon, either before the cells separate or soon afterwards, the protoplasm collects at the poles, i. e. the nucleus divides directly, and stage 1 is reproduced. The division of the cell in stage 1, and the formation of stage 3, have been directly observed.

Dr. Delépine, who kindly examined some of the specimens, suggested that some of the other appearances were due to karyokinesis, and, having regard to the columnar form of the organism and its small size, various forms may be distinguished which would represent the formation of a central plate and its subsequent division and gradual separation. These forms are represented in fig. 4, *b*. Starting with a cell, whose contents stain equally and moderately, these contents representing a nucleus, there is a gradual gathering of the chromatin until an intensely staining central plate is formed with unstained



poles. The whole cell is at the same time larger. The central chromatin then shows signs of division, and the two halves gradually separate and travel away from one another towards the poles. Division of the cell takes place between the two halves, and the chromatin is once more equally distributed over the cell. The appearance of constriction in fixed and stained specimens at the central plate is partly artificial, as in organisms examined in a drop culture containing methylene blue the outer border of the cell may be made out, preserving its original breadth until the division is about to take place. It has already been mentioned that the *B. typhosus* presents similar appearances to the above, and Babes ('Soc. Anatomique,' December, 1884) figures and describes very similar forms occurring in the comma bacillus of Koch. He states that at first the poles of the cells are deeply stained, but as the organism grows the deeply staining portions pass to the centre and become fused; and that then a clear space forms, dividing the central mass into two parts, which indicates the commencing cell division.

This organism, when grown under somewhat unfavourable conditions, readily shows involution forms. When grown on gelatine with scanty access of air the cells become three to four times as long as broad without, in many instances, dividing, and after some days become vacuolated. These vacuoles are generally three in number, and symmetrically arranged. This vacuolation explains many of the appearances seen in the stained specimens. The cultures in fluid media, especially in the depths and in sugar-containing fluids, show long, almost leptothrix, forms, and there is a great tendency to the formation of bud-like projections, which in some instances are so prolonged as to resemble branchings. The protoplasm in these forms also presents irregularities of distribution.

The following is a short list of the principal red pigment-forming bacilli, with their differential characteristics:

| <i>Name.</i>                        | <i>Character of Cells.</i>                                             | <i>Culture on Gelatine.</i>                               |
|-------------------------------------|------------------------------------------------------------------------|-----------------------------------------------------------|
| <i>M. prodigiosus</i> .             | Oval and round cells .5 to 1 $\mu$ , also has a bacillus form. Motile. | Liquefies gelatine quickly.                               |
| <i>B. ruber</i> (Frank) .           | Bacilli from 6 to 8 $\mu$ long, by 1 $\mu$ broad. Very motile.         | Liquefies gelatine.                                       |
| <i>B. rouge de Kiel</i> (Breunig)   | Bacilli 2.5 to 5 $\mu$ long, by 7 to 8 $\mu$ broad. Motile.            | Liquefies gelatine, which is rapidly coloured throughout. |
| <i>B. indicus</i> (Koch)            | Short cells with rounded ends.                                         | Liquefies gelatine.                                       |
| <i>B. granulatus roseus</i> (Babes) | Straight rods 3 to 4 $\mu$ broad, 5 to 6 times as long.                | Does not liquefy gelatine.                                |
| <i>B. corallinus</i> . .            | Oval cells and rods 1 $\mu$ broad, 2 to 3 $\mu$ long.                  | Does not liquefy gelatine.                                |

### DESCRIPTION OF PLATE XXXI,

Illustrating Mr. Charles Slater's paper "On a Red Pigment-forming Organism, *B. corallinus*."

FIG. 1.—Organism unstained.

FIG. 2.—Organism stained alkaline methylene blue.

FIG. 3.—Organism stained logwood, showing apparent spore-like bodies.

FIG. 4.—(a) Stages indicating direct division. (b) Forms possibly due to karyokinesis.

FIG. 5.—Budding and branched forms occurring in liquid media.

FIG. 6.—Vacuolated forms unstained.

FIG. 7.—Involution forms—torula-like forms.

FIG. 8.—Organism from fluid, sugar-containing medium; stained.

FIG. 9.—Organism growing in gelatine medium.

FIG. 10.—The same colonies twenty-four hours later, showing growth and cell division.

Figs. 9 and 10 are not so highly magnified as the others.

FIG. 11.—Potato culture.

## Immunity against Microbes.

By

**M. Armand Ruffer, M.A., M.D.(Oxon.).**

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With Plates XXXII and XXXIII.

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### PART II.

THE first part of this paper, with few exceptions, was taken up with the struggle taking place in the healthy body between micro-organisms and amœboid cells. It is now time to study what happens where micro-organisms have found their way into the tissues of animals.

Very little consideration, however, shows that the problem to be solved is a most difficult one, on account of the many factors which influence the clinical course and pathological appearances of a disease.

In the first place, the clinical aspect and the pathological appearances will vary according to the number of micro-organisms introduced into the system. This fact was formerly denied by competent bacteriologists; for it was difficult to imagine that the number of micro-organisms could be of importance, when it was shown that 1,000,000,000,000th part of a drop of blood from an animal dead of septicæmia injected into another animal reproduced the same disease. Lately, however, overwhelming evidence has been brought together proving the influence of numbers. Thus 250,000,000 staphylococci injected into a rabbit will produce an abscess in this animal,<sup>1</sup> but 1,000,000,000 of the same parasites will cause

<sup>1</sup> Davaine.

death.<sup>1</sup> A few drops only of a culture of the *Bacillus pyocyaneus* injected into the veins of a rabbit give rise to a chronic disease; whereas a quarter of a cubic centimetre of the same culture kills the animal with certainty.<sup>2</sup> One cubic centimetre of an emulsion of the culture of the *Bacillus prodigiosus* in beef-broth produced no effect on a rabbit;<sup>3</sup> whereas 3 cubic centimetres of the same emulsion proved fatal to another animal.

Another and most important factor is the virulence of the micro-organisms introduced into the system. Without entering at present into the question of the means by which the virulence of microbes can be raised or lowered, or into the other question as to what is really meant by virulence, it is a well-ascertained fact that this factor varies within very wide limits. The same number of anthrax bacilli contained in a drop of blood from an animal dead of anthrax, in a drop of Pasteur's first or of his second vaccines for the same disease, gives rise to widely different effects if introduced into three animals of the same species. The disease produced in rabbits by the injection of a culture of the bacillus of fowl cholera ten days old differs widely from that produced by the injection of a culture one day old. One might say almost that the virulence of a given species of micro-organism is inversely proportionate to the number of microbes necessary to kill a given animal.

The species of animal used for the experiments is, perhaps, of still greater importance than the quality of the virus. Rabbits inoculated with the bacilli of fowl cholera, of anthrax, or of diphtheria, speedily succumb to these diseases, but the injection of cultures of quarter-evil or typhoid fever has no effect on these same animals. Guinea-pigs, on the other hand, perish quickly when the bacillus of quarter-evil is injected into them. The inoculation of the bacillus of anthrax into a number of European sheep is followed by the death of

<sup>1</sup> Odo Bujwid's numbers are somewhat higher, but this does not alter the truth of the fact.

<sup>2</sup> Charrin, 'La Maladie Pyocyannique,' Paris, 1889.

<sup>3</sup> M. Armand Ruffer, unpublished observations.



most of them, whereas Algerian sheep are not affected by the same virus. The virus which may prove fatal to puppies proves harmless to full-grown dogs.

The result of the inoculation is influenced to a considerable extent by the fact of the animal having recovered from a previous attack of the same disease, or having been rendered immune against it by artificial means; much will also depend on the state of the animal's health at the time of inoculation. Fowls which are naturally immune against anthrax take the disease readily if their temperature be artificially lowered by immersion into cold water. Contrariwise, frogs, which are also immune against anthrax, inoculated with the same disease, die when their temperature is artificially raised. Moreover, if the temperature of a warm-blooded animal be artificially lowered, or if it be submitted to long-continued fright, the micro-organisms normally present in the intestine pass through the lining epithelium and invade the blood.<sup>1</sup>

Lastly, much will depend on the mode of introducing the virus. Thus, a quarter of a cubic centimetre of a culture of *Bacillus pyocyaneus* injected into the veins of a rabbit proves fatal within forty-eight hours; but the same quantity injected under the skin causes death after a week only, or not at all.<sup>2</sup>

The problem is a very complex one, and in this paper, therefore, the writer will only make use of data which have been obtained by actual experiments.

It will be best to discuss in the first place the cases in which the virus produces a well-marked inflammatory reaction at the point of inoculation, when introduced under the skin. We will take, as examples, the cases in which guinea-pigs have been inoculated with virulent quarter-evil (*Bacillus Chauvæi*), as being the most typical example of the kind.

At the end of twelve to eighteen hours after a small quan-

<sup>1</sup> Ch. Bouchard, "Essai d'une Théorie de l'Infection," 'Proceedings of the Tenth Medical Congress,' Berlin, 1890.

<sup>2</sup> Charrin, 'La Maladie Pyocyane.' Compare also Armand Ruffer 'On the Nature of the Disease produced by the Inoculation of the *Bacillus pyocyaneus*.'

tity of the virus, in the shape of a dried powder, has been introduced under the skin, a small hard swelling is formed at the seat of inoculation, which crepitates on pressure as if its contents consisted of gas and fluid. The tumour increases in size, and soon becomes harder and more resistant. The parts surrounding it are then considerably thickened, the walls of the abdomen, for instance, feeling twice as thick as natural. After twenty-four hours or so the animal shows evident signs of a general illness; it refuses food, does not run away when touched, gets more and more drowsy, and generally dies just within forty-eight hours after the inoculation.

On examining the tissues of the spot where the inoculation has been made, the remains of the powder containing the virus are found lying in a kind of cavity full of almost clear fluid. The walls of this cavity are lined by a thin, greyish, and fibrinous layer. The muscles surrounding the cavity are highly œdematous, so that on cutting through them a large quantity (10 c.c. to 20 c.c. or more) of clear, transparent, reddish fluid may be collected.

If twelve hours after the inoculation a sharp capillary pipette be introduced into the centre of the tumour already formed, and a little of the transparent fluid be carefully drawn off and examined under the microscope, an enormous number of bacilli will be found moving about in the liquid. Here and there a leucocyte is seen. As a rule, the wandering cells met with at this stage are empty, but occasionally one holds two, three, or more bacilli in its interior.

In cover-glass preparations stained in an alkaline solution of methyl-blue, or better, gentian-violet, the bacilli floating in the liquid are, to all appearances, perfectly normal, and, according to the reagent used, stain of a lovely dark blue or purple colour. Most of the bacilli contained in the cells stain normally, but here and there an intercellular bacillus shows signs of degeneration.

A drop of the liquid from the tumour withdrawn and examined at the end of forty-eight hours, or immediately after the animal's death, contains an enormous number of free

bacilli. The fluid, as a rule, is somewhat turbid, owing to the presence of wandering cells in fairly large numbers. These amœboid cells belong to the small variety (microphages), and are either uni- or multi-nucleated. The nuclei possess hardly any intra-nuclear network, and are surrounded by a small amount of protoplasm only. Many of these cells contain bacilli, some in enormous numbers, as many as ten micro-organisms being occasionally enclosed in one cell. The micro-organisms in the liquid show no signs of degeneration, whilst some of the bacilli contained in the amœboid cells have clearly undergone a process of digestion. Floating in the liquid are seen remnants of the dried blood used for the purpose of inoculation.

On microscopical examination the free aspect of the abscess-wall is covered everywhere by an enormous number of bacilli, lying free in the coagulated exudation fluid, staining in a perfectly normal manner, and showing to all appearances no traces of degeneration.

The abscess-wall itself consists of an innumerable number of small migrating cells. Some of these amœboid cells have but one single nucleus; others contain two, three, or four nuclei. In many the nucleus has the triradiate aspect found in other inflammatory conditions—for example, pneumonia. The amount of protoplasm round the nucleus is very small as a rule. Close to the free surface of the abscess-wall these cells are pressed together in enormous numbers, and so closely packed that the contours of each are scarcely to be recognised.

In the deeper regions of the abscess-wall the cells are massed together less closely. The muscular tissue around contains within its meshes and between its fibres a large number of small round migrating cells. The latter are met with even in places where not a single bacillus is to be seen. Here and there, especially in the deeper strata of the abscess-wall, a few cells occur which are larger, have a single, clear, vesicular nucleus, with a well-marked intra-nuclear network, surrounded by a large amount of coarse protoplasm, and not infrequently contain in their interior remnants of degenerated bacilli and



leucocytes. These cells probably represent the first stage in the development of macrophages; they do not attain their full size owing to the early death of the animal. On the whole, macrophages play quite a secondary part at this stage.

Many leucocytes found close to the free surface of the abscess-wall contain in their interior a large number of characteristic bacilli. Nevertheless, many micro-organisms remain quite free between the cells in the surrounding medium, which has been coagulated by the action of the hardening reagent; but whereas the latter micro-organisms do not show signs of degeneration, many of those contained in the migrating cells have undoubtedly undergone a process of disintegration. The number of micro-organisms in the deeper layers of the abscess-wall, at some distance from the free surface, gradually becomes smaller and smaller; and while in the upper strata some micro-organisms are free between the cells, the bacilli in the lower strata of the abscess-wall are always contained in the interior of macrophages. Lastly, a few millimetres away from the free surface, although the number of migrating cells is still very large, no bacilli are to be seen except occasionally the remnant of a dead micro-organism contained in an amœboid cell.

It will be convenient to speak now of the forms of degeneration as seen in the micro-organisms of quarter-evil, when they have been taken into the interior of lymphocytes. I will describe these changes as seen in preparations examined with a Vêrick microscope, oil immersion  $\frac{1}{12}$ th or  $\frac{1}{13}$ th, ocular 1 or 3, and Abbé's condenser. The bacilli not contained in the cells I shall, for brevity's sake, call extra-cellular micro-organisms, whilst the bacilli in the interior of leucocytes must go by the name of intra-cellular micro-organisms.

The extra-cellular micro-organisms present in the coagulated exuded fluid, when carefully examined, consist of rods somewhat shorter and thicker than anthrax bacilli. Spores are not often present when the virus is inoculated into guinea-pigs. The bacilli stain of a deep blue colour with methyl-blue, intensely red with fuchsin, and purple with gentian-violet.

Though staining uniformly, the extra-cellular bacilli are not



all of the same length and thickness; these differences in size being due partly to an actual difference in size, partly to two or more being joined together, and possibly to the fact that some are placed more or less obliquely in the preparation. None show any signs of degeneration whatever.

The appearance of the intra-cellular bacilli is very different. A large number are, to all appearances, quite healthy, stain uniformly, and are of normal size or shape. Here and there, however, an intra-cellular bacillus is found which, instead of staining as deeply as the others, assumes a light purple tinge. This tint may be perfectly uniform, but at other times part only of the bacillus is stained of a dark purple colour, whilst other parts of the same micro-organism assume a lighter shade, and the most varied forms of degeneration can be observed.

Sometimes the centre of the bacillus takes up the colouring matter badly, whilst the periphery still stains deeply. The micro-organism then consists of a central, badly staining core, and a dark, deeply staining sheath (see figs. B 3, *b*, B 5, *c*). Sometimes one edge only of the micro-organism retains the colouring matter deeply, whilst the remainder assumes a light purple colour (fig. B 5, *b*). In another stage the whole bacillus is of a uniform pale purple hue, owing to the gradual breaking up and disappearance of the colouring matter in the sheath.

In other bacilli the degeneration, marked by the loss of power of fixing the colouring substance, begins at the periphery of the micro-organism, the centre still retaining the colouring matter, while the edges remain unstained. The central coloured part becomes less and less in amount, so that at last a small streak of it only is left. Later on the central part itself breaks up, and forms a very thin, interrupted, irregular streak lying in the interior of a bacillus.

The process of degeneration in other bacilli affects the whole breadth of the bacillus uniformly. One end of the bacillus stains normally, whilst the other is of a pale homogeneous colour (see fig. B 4, *b*). Sometimes an intra-cellular bacillus consists of a row of dots staining darkly, and embedded in a

less darkly staining material (fig. A, *a*), or of dark and light parts alternately (see fig. B 3, *a*).

At the same time that these changes take place in the colouring of the bacilli, the latter undergo alterations in shape and size. Whereas the bacilli present in the inflammatory fluid are usually straight, and very rarely bent, many of the intra-cellular bacilli are curved on themselves (see figs. A and B). This is better seen in sections made at the point of injection in animals inoculated with a weak virus, in which, as we have seen, the bacilli have a tendency to join together end to end. These intra-cellular rods are nearly always curved, and present a highly characteristic appearance (figs. A, B).

The intra-cellular bacilli in later stages of degeneration show a diminution in thickness. Many are much thinner than normal (fig. B 2, *a, b*); and while the diminution of thickness in some bacilli is uniform, in others it begins at one extremity, the bacillus looking as if one end of it were being eaten away (fig. B 3, *c*). The contours of the intra-cellular micro-organisms, also, instead of being sharply defined as in the extra-cellular bacilli, are irregular, and their exact outline is by no means easily traced out (see figs. A and B 3, *a*). In a later stage the bacilli become thinner and thinner, more and more irregular (fig. B 2, *a, b*), and lose whatever power they still possessed of retaining colouring matter, so that finally nothing is left in the cells but small dots (figs. A, *c, d, e*, and B 1, *a*), some of which still retain more or less colouring matter, whilst others appear as light, highly refracting granules, or very pale rods, looking like the remnants of the sheaths of dead bacilli.

Occasionally intra-cellular bacilli are met with which, instead of taking up aniline dyes, stain with carmine or logwood. Others stain partly with carmine and partly with aniline dyes, looking like little dots stained purple and red alternately.

When a small dose of a weak virus is inoculated subcutaneously, the struggle between leucocytes and bacilli is evident on the fifth day, or even later; but the number of micro-organisms gradually diminishes, so that after the fourth

day hardly any bacilli are found at the seat of inoculation. On the fifth day the few bacilli which can still be seen are contained in migrating cells, and show evident signs of the most advanced degeneration.

The study of the initial lesion of animals in which a chronic form of the disease has been induced by the inoculation of large quantities of a weak virus (0·40 centigramme or more) is not less interesting.

The animals inoculated with a strong virus perish so soon after the introduction, that the struggle between the cells and the micro-organisms is more or less one-sided; for although the animal dies, most of the cells present at the point of inoculation are normal and show no signs of degeneration. When, however, a chronic form of the disease is produced, many of the lymphocytes perish as the result of their struggle with the invading bacilli.

If the animal dies on the fourth or fifth day, sections through the exact spot where the virus has been inoculated show that the bacilli have infiltrated the neighbouring muscles to a far greater extent than in the acute form of the disease. Many bacilli are extra-cellular, lie in a coagulated inflammatory effusion, and are perfectly normal and healthy, staining well with aniline dyes, and retaining colouring matters in a normal fashion. A few micro-organisms are contained within amœboid cells, and often present various forms of degeneration; but although in the acute form of the disease most of the wandering cells are healthy, many of the cells met with in the more chronic affection are markedly degenerated. In other words, they become true pus-cells—that is, dead cells.

The nucleus of such a cell, instead of staining deeply with carmine or logwood, possessing a coarse intra-nuclear network, and being marked off sharply from the surrounding protoplasm, stains rather more diffusely, and often shows signs of breaking up. In later stages the nucleus undergoes distinct fragmentation, three or four fragments of nuclei lying in the protoplasm of a cell. In the last stage the nucleus disappears, nothing remaining but a pale round mass of protoplasm, which no



longer takes up colouring matter. Not infrequently one of these cells is contained in a larger cell, possessing a clear vesicular nucleus, which is surrounded by a large amount of somewhat coarse protoplasm; in other words, it is taken into the interior of a typical macrophage.

Some of the dead lymphocytes contain bacilli which sometimes appear healthy, but not infrequently are more or less degenerated. It might be supposed that these cells died owing to the entrance of bacilli into their protoplasm; many degenerated cells, however, are found in which not a single living or dead bacillus is to be seen. These cells probably never contained bacilli, but perished as the result of the toxic influence of the poisons secreted by the micro-organisms surrounding them. In the chronic form of the disease, therefore, it is possible to follow the struggle of the bacilli and the lymphocytes, a struggle in which sometimes the former and sometimes the latter perish. It is a noticeable fact that although the animal dies from the inoculation, no micro-organisms penetrate into the tissues; the bacilli have all been arrested at the point of inoculation, and this arrest is evidently due to the barrier formed by the amœboid cells at the seat of inoculation.

Similar facts have been observed in the erysipelas of man, or when the disease is artificially produced in animals by inoculation.

The part of the skin affected with erysipelas may be divided into three zones. The first or peripheral zone, although showing no trace of redness, nevertheless contains numerous streptococci lodged in the lymphatic vessels. The second zone looks rather inflamed, and contains many migrating cells, the greater number of which are filled with streptococci. Finally, the third or innermost zone is crowded with small round-cells, and contains no streptococci.

The following facts were observed on seven cases of erysipelas. Two of these cases proved fatal, and in them numerous streptococci were found in the lymphatic vessels, in the skin, and in the layers immediately beneath, but always outside the vessels.



The cocci were occasionally single, more often they formed a more or less lengthy chain. Innumerable leucocytes had emigrated to the spot, whilst the fixed cells of the connective tissue showed signs of proliferation.

The microscopic appearances were very different in the cases which ended in recovery, for many of the migrating cells were absolutely crammed with micro-organisms, forming a mass surrounded by a clear vacuole in the interior of the leucocyte. The nucleus of such leucocytes varied greatly in size, and was irregular in shape and not easy to stain. The leucocytes were far less numerous where the skin had become gangrenous, and nearly all the streptococci were then outside the cells.

Other amœboid cells larger than leucocytes were also present. Their nuclei, instead of staining deeply with methylene blue, took the stain badly, and they further differed from those of leucocytes by their oval and irregular contours, and by their possessing very distinct nucleoli. These cells also belonged to the group of phagocytes.

The same phenomena may be noticed in white mice inoculated with erysipelas. If little cylinders of elder-pith, previously soaked in a pure culture of streptococci, be placed under the skin of such animals, the foreign body very soon becomes surrounded by a mass of leucocytes. The latter are so full of streptococci that the cells stand out as black dots in sections stained with gentian-violet. Two days afterwards almost all the streptococci have disappeared. But in the mouse, as in man, not a single one is to be found in the macrophages. The micro-organisms enclosed in the leucocytes gradually lose their regular outlines, break up and disappear, and when stained with methylene blue, may assume any shade of colour, from an intense blue to a reddish purple or pale violet.

Another series of researches, made with the bacillus of "rouget des porcs" or swine fever,<sup>1</sup> has yielded similar results. According to Emmerich and Mattei,<sup>2</sup> the bacilli of swine fever

<sup>1</sup> E. Metschnikoff, "Études sur l'Immunité," 'Annales de l'Institut Pasteur,' June, 1889, p. 289.

<sup>2</sup> Emmerich and di Mattei, 'Vienna Congress,' 1888.

injected into rabbits previously rendered immune against this disease are completely destroyed within fifteen to twenty-five minutes after their introduction, even if the quantity of culture injected be very considerable. If this be correct, it is impossible to ascribe the destruction of micro-organisms to the action of phagocytes ; for, in order to exert their action, phagocytes must first accumulate round the injected culture, then take the bacilli into their interior, and finally destroy them. According to these authors<sup>1</sup> this destruction is accomplished by an antiseptic liquid which is not produced by the bacilli, but by the cells of the immune organism.

Metschnikoff, however, who controlled the facts published by Emmerich and di Mattei, has obtained very different results from those published by these observers.

Metschnikoff inoculated rabbits with the bacilli of swine fever, and after a lapse of time, varying from half an hour to six days, removed with a capillary pipette one or several drops of liquid which had accumulated at the point of inoculation. This liquid was sometimes quite transparent, but more often was slightly stained with blood, and in several cases consisted of almost pure blood.

In four cases the liquid taken from the point of inoculation 6 $\frac{3}{4}$ , 17, 19, and 26 hours after the operation, contained no micro-organisms. In eleven other cases, in which the liquid was withdrawn 1 $\frac{1}{2}$ , 4, 5, 6, 6 $\frac{3}{4}$ , 19, 20, 24 hours, and 4 days after the inoculation, the beef-broth into which it was placed gave pure cultures of the bacilli of swine fever, which, when inoculated into pigeons and mice, always proved exceedingly virulent. The liquid collected at the point of inoculation 4, 19, 20, and 24 hours after the injection of the first vaccin into both immune and non-immune rabbits, contained bacilli of the first vaccin. The liquid taken 19 hours after the inoculation of two animals, which had been rendered immune against swine fever by repeated injections, gave a culture ; whilst a third sample, taken 20 hours afterwards, remained sterile.

<sup>1</sup> 'Fortschritte der Medicin,' 1888, T. vi, p. 729.

Further, Metschnikoff proved that the bacilli of the first vaccin of swine fever live a long time in the anterior chamber of the eye of rabbits, as well as in the eye of dogs, which are naturally immune against swine fever.

One rabbit, after having been rendered immune by seven injections of the virus, was finally inoculated into the anterior chamber of the eye with the first vaccin. In spite of the previous inoculations, a large number of leucocytes emigrated into the anterior chamber. A drop of aqueous humour examined twenty-two hours after the operation gave a pure culture of the first vaccin, whilst another drop, taken after forty-eight hours, contained no bacilli. The bacilli of the first vaccin were generally contained in the interior of leucocytes which had found their way into the anterior chamber.

A small case composed of four cover-glasses joined together with sealing-wax, and filled with the virus of swine fever, was introduced under the skin of a rabbit. One hour after its introduction the case was already full of leucocytes containing bacilli, which stained quite as well as those lying in the exudation fluid; on cover-glasses withdrawn two and a half hours after their introduction under the skin of a rabbit, which had resisted six former injections of the virus, the number of leucocytes containing bacilli in their interior was considerable. In all M. Metschnikoff's experiments with cover-glasses the bacilli were very quickly taken into the interior of wandering cells, which gradually became more and more numerous at the point of inoculation.

A glass-case was filled with the virus of swine fever and left for fifty-one hours under the skin of an immune rabbit. It was then found that almost all the bacilli were contained in the interior of cells and showed marked signs of degeneration; their outlines had lost their customary clearness, stained badly, and their contents had become granular. On the other hand, it was found that in glass-cases placed under the skin of non-immune rabbits the greater number of the bacilli, although surrounded by phagocytes, appeared perfectly normal and stained well.



In order to see exactly what does happen when micro-organisms are inoculated into a living fluid containing no cells, Metschnikoff<sup>1</sup> introduced the spores and bacilli of anthrax into the anterior chamber of the eye of living animals. In this manner he proved that even in animals like frogs, which are naturally immune, or animals rendered artificially immune, e. g. sheep, the aqueous humour is a very good cultivating material for anthrax. The micro-organisms (even those of the first vaccin) inoculated into the anterior chamber of the eye of immune sheep, grow and multiply rapidly until arrested by leucocytes, whilst the same micro-organisms inoculated into the subcutaneous tissue of the same animal do not multiply. The normal aqueous humour of immune animals, therefore, contains no substances harmful to the bacillus of anthrax.

Anthrax spores inoculated into the anterior chamber of the eye of rabbits multiply abundantly, and cause the death of the animal. In order to isolate the virus from cellular influences it may be placed in small cylinders of elder-pith, or in a small bag prepared from the intestines of frogs, or more simply still, in filter-paper. The spores thus protected, when introduced under the skin of frogs, grow into normal bacilli, whilst the unprotected germs are devoured by amœboid cells. On introducing under the skin of frogs membranous bags containing blood of guinea-pigs which had succumbed to anthrax, the bacilli grew abundantly and preserved their virulence until the sixth day at least; it is clear, therefore, that the living fluids of frogs have no harmful influence on anthrax. Conversely, the living unprotected bacilli are eaten by the cells of frogs.

Very interesting also are the facts observed when anthrax is inoculated into rats. Young white rats<sup>2</sup> succumb to anthrax much more readily than adult ones, and by passing the virus

<sup>1</sup> E. Metschnikoff, "Sur la Manière d'être des Bactéries Charbonneuses dans l'Organisme," 'Virchow's Archiv,' Dec., 1888 ('Annales,' Jan., 1889, p. 41).

<sup>2</sup> E. Metschnikoff, "Études sur l'Immunité," 'Annales de l'Institut Pasteur,' April, 1890, p. 199.



through several rats it is possible to obtain an exceedingly powerful virus, which proves fatal to very old rats in three to six days after inoculation.

It happens occasionally that the bacilli of anthrax will not grow in vitro in the blood of rats which have actually died of anthrax; but, as a general rule, the spores of bacilli thrive in the blood of rats, which, before being killed, proved immune against anthrax. The bacilli of anthrax grew abundantly and produced spores even in the aqueous humour of rats which had survived an inoculation of anthrax. The same holds true for the subcutaneous tissue, which often contains numerous leucocytes.

Spores of anthrax inoculated into rats always germinate and produce bacilli, no matter whether the inoculation be performed into the anterior chamber of the eye or under the skin. One such experiment is extremely interesting, and may be related here. A white rat was examined four days after having been inoculated subcutaneously with the blood of a guinea-pig dead of anthrax; there was considerable œdema at the point of inoculation, but the exudation fluid contained degenerated bacilli only. Several little silk threads loaded with anthrax spores were introduced into the œdema, and twelve hours afterwards a considerable number of young and perfectly normal bacilli were found in the fluid. Eighteen hours after the introduction of the threads one was withdrawn, and found to be surrounded by an immense number of bacilli, staining easily, and perfectly normal in every respect. The rat survived the two inoculations, and only died three months after from the effects of an inoculation in the eye, with moderately virulent anthrax.

The inoculation of anthrax into the most resistant rats is usually followed by an abundant growth of anthrax bacilli; but after repeated inoculations on the same animal the latter becomes so perfectly immune that finally the spores do not seem to grow at all. M. Metschnikoff inoculated an old rat (female) which had withstood three previous injections of anthrax, by introducing under the skin of the back a silk thread

laden with spores, and wrapped in a little piece of sterilised cotton-wool, another similar thread being placed on the cotton-wool without being surrounded by the latter. Nineteen hours afterwards a semi-transparent liquid exudation had gathered round the threads, and a few short but normal bacilli could be distinguished in it. The thread lying in the cotton-wool was surrounded by far more anthrax bacilli than its fellow outside thread. These prove, then, that anthrax bacilli find a medium favorable to their growth even in animals rendered immune by previous inoculations, and that they grow best when protected against the attacks of amœboid cells. Further, bacilli of the first vaccin may develop in the organism of rats which have resisted virulent anthrax—in the anterior chamber of the eye of a white rat one month and a half after its recovery from virulent anthrax, for instance.

Some of the leucocytes of white rats inoculated with anthrax contain no, or very few, bacilli; whilst others devour them in such numbers that it is often difficult to distinguish individual bacilli. These greedy phagocytes are with but few exceptions multinucleated microphages. The bacilli contained in these cells often stain easily, and appear normal; but, on the other hand, more or less degenerated micro-organisms are often met with.

When filled with bacilli phagocytes burst with extraordinary facility, and in every field the observer meets with cells which have allowed part of their contents to escape. The presence of free and degenerated bacilli in the exudation of white rats inoculated with anthrax, which at once strikes the observer, can thus be readily explained. Metschnikoff, however, does not think that all the bacilli found dead outside the cells come without exception from the interior of phagocytes, for, in the most favorable cultivating media even, a certain number of microbes die, either as a result of the sudden transportation into a new ground, or from some similar cause.

During the first two days immediately following the inoculation of anthrax into the anterior chamber of the eye of white rats the larger number of the bacilli are found outside the cells;

but on the third day, should the rat recover, all of them almost are taken into the interior of leucocytes. Amongst the intracellular bacilli a great many for a time retain the property of absorbing aniline dyes; others lose this property, and only stain very slightly. Many of the leucocytes, however, perish, as is proved by the granular appearance of their nuclei. Besides the débris of the nuclei of these phagocytes, more or less degenerated extra-cellular bacilli are sometimes found, which at some time were undoubtedly in the interior of leucocytes. The exudation from the anterior chamber of the eye also contains a more or less large number of macrophages, which, however, do not as a rule destroy many bacilli, but often enclose a large number of leucocytes.

The following experiment shows that the bacilli are taken into the interior of leucocytes whilst still in the living state. A drop of exudation was removed from the eye of a rat inoculated with anthrax after the death of the animal, and at a time when the phagocytes contained a great number of rods; and after ascertaining that all the leucocytes were really dead a little broth was added to the exudation, and the large drop thus produced placed in the warm chamber. Some hours later it was plain that a certain number of the bacilli were alive in the interior of the leucocyte, for they had multiplied and lengthened into filaments. A drop of this exudation introduced under the skin of guinea-pigs produced fatal anthrax in these animals.

Within the tissues and internal organs of white rats anthrax bacilli are very soon taken into the interior of microphages, or, more frequently, macrophages, e. g. the cells of the splenic pulp, the star-shaped cell of Kupffer, and the mononuclear leucocytes found in the capillaries of the liver. As the macrophages get more and more filled with bacilli the vacuoles increase in number, and the protoplasm at last becomes a mere sac with thin walls, filled with a transparent substance, evidently formed by the secretions of the phagocytes and of the parasite. The nucleus, which keeps its characteristic shape is found in the thickest part of the sac.



The macrophages filled with bacilli and cells, swollen in consequence of the formation of large vacuoles, finally burst and allow their contents to escape. The bacilli, freed in this way, often show signs of degeneration. Thus one meets with free bacilli, segments of which stain violet and pink alternately.

Confirmatory evidence has been obtained by Metschnikoff by studying the processes taking place when pigeons are inoculated with anthrax. This animal is immune against the disease, and Baumgarten<sup>1</sup> and Czaplewski state that anthrax bacilli introduced under the skin of pigeons perish spontaneously at the end of four hours, the phagocytes taking no part in their destruction. Lubarsch also came to the same conclusion.

Nuttall, on the other hand, thought that the blood of pigeons is a medium in which the bacilli of anthrax begin to grow almost immediately after their introduction into it.

Metschnikoff<sup>2</sup> then proved that anthrax does not become attenuated in pigeons, but often acquires new virulence.

The aqueous humour of pigeons introduced into capillary tubes and inoculated with spores of anthrax proves a most satisfactory cultivating medium, the spores giving rise to an abundant crop of felted threads, which are perfectly normal in appearance and ultimately produce innumerable spores. The same result is obtained when anthrax is inoculated into the aqueous humour of the eye of a pigeon which has been rendered doubly immune by a previous inoculation of anthrax from which it has recovered.

The same result can be obtained by inoculating the exudation fluid with an exceedingly small quantity of an old culture of the first vaccin of anthrax. The day after the inoculation the whole drop is full of an abundant culture of normal anthrax filaments.

The following experiment is extremely interesting :—A small piece of silk thread loaded with spores was introduced into the anterior chamber of the eye of an immune pigeon inoculated

<sup>1</sup> 'Centralblatt für klinische Medicin,' 1888, No. 29, p. 516.

<sup>2</sup> Metschnikoff, "Études sur l'Immunité: Le Charbon des Pigeons," 'Annales de l'Institut Pasteur,' 1890, vol. iv, p. 65.



two days previously with spores of anthrax in the same eye. Before introducing the thread it was ascertained that the aqueous humour of the eye contained a considerable number of leucocytes, but no free bacilli; the day after, on withdrawing the thread, it was seen that a certain number of spores had grown into bacilli composed of several (as many as six) segments. In another similar experiment the piece of silk thread was introduced five days after the first inoculation, and at a time when the bacilli had already entirely disappeared. Twenty-four hours afterwards newly grown bacilli, showing as many as seven segments, were found in the exudation from the eye. The bacilli took a dark blue colour with methylene-blue, and were quite normal.

Repeated subcutaneous inoculations into pigeons which had already recovered from one attack of anthrax no longer gave rise to serious symptoms. Nevertheless the bacilli introduced in the state of spores fixed on silk threads grew more or less abundantly.

Four hours only after the subcutaneous inoculation of very virulent anthrax into pigeons a marked emigration of microphages took place at the point of inoculation, and some of these contained one or more and as many as nine bacilli.

After a time the inflammation increased, and a larger number of phagocytes—micro- and macro-phages—accumulated at the point of inoculation. Many phagocytes, especially those which were crammed with bacilli, burst very easily, allowing their contents, including micro-organisms in every stage of degeneration, to escape. This bursting took place not as a consequence of the mode of preparation, but also in the living animal—as was proved by the degenerated form of some phagocytes containing bacilli.

In pigeons which recovered from the disease the larger number of the bacilli were found in the interior of the phagocytes, whilst in the birds which perished the majority of microbes lay outside the cells, although in all, the amœboid cells devoured a large number of micro-organisms. So full were the microphages sometimes that their nuclei could hardly be made out, and when

in that state they might easily be mistaken for clusters of bacilli.

Anthrax does not become less virulent by passing through pigeons. Thus, a guinea-pig contracted anthrax when inoculated with virus which had remained six days under the skin of an immune pigeon; and a large rabbit died after being inoculated with a drop of exudation extracted twenty-two hours after the introduction of anthrax blood into the eye of a pigeon which had undergone four previous inoculations. A guinea-pig received one drop of exudation taken twenty-six hours after the inoculation of anthrax into the eye of a pigeon; it is noteworthy that the number of free bacilli in this exudation fluid was already greatly diminished, and that the leucocytes contained masses of bacilli; nevertheless the guinea-pig contracted the disease, and died from it sixty hours after the inoculation.

The following beautiful experiment shows that the cells take into their interior living micro-organisms. A drop of exudation fluid was removed from the anterior chamber of the eye of an animal inoculated with anthrax. This, being found to contain numerous cells enclosing bacilli, was mixed with a very small quantity of beef broth, and the whole was placed in a warm chamber. The phagocytes died at once, but the bacilli continued to grow; and by placing under the microscope some cells enclosing bacilli, the development of the latter could be easily followed.

After three hours' stay in the warm chamber it was easily ascertained that the bacilli contained in several of the phagocytes had already begun growing. Three of these phagocytes were isolated by a difficult but very neat process, and thrown into beef broth; the development of these bacilli was carefully watched, and it was ascertained that they were alive and active. At the end of eight hours the bacilli in the drop gave a culture containing filaments, and the next morning an abundant pure growth had developed. A white mouse, a young guinea-pig, and two full-grown rabbits were inoculated with this culture; the mouse died in twenty hours, and the guinea-pig twenty-four hours after the inoculation, while one of the

rabbits died in four days and the other in less than three. The four animals showed the characteristic signs of anthrax (bacilli in the blood and organs, hypertrophy of the spleen, œdema of the skin, in the rabbit and guinea-pig, &c.).

Similar facts<sup>1</sup> have been observed in fowls when these animals which are immune against anthrax are inoculated with this virus. When, however, these animals are placed in cold water they readily die when inoculated with anthrax, and Wagner has shown that the phagocytes in the latter case are unable to cope with the bacilli, though they may make an attempt to do so.

I have shown elsewhere that in diphtheria of man a struggle takes place in the diphtheritic membrane between the leucocytes and the specific microbes, and that the disease is localised in the upper part of the respiratory tract owing to the action of the former. In thin sections passing through the entire false membrane and the surrounding structures—a section of a small bronchus, for instance, when the disease has extended into the lungs—the most superficial part of the membrane, that is the free edge of the lumen of the tube, is found to contain an enormous number of specific bacilli, lying, for the most part, quite free in the exudation fluid (fig. C). The micro-organisms resemble short rods of the size of tubercle bacilli, and are often aggregated together in clusters containing hundreds of microbes. So thick is this layer of micro-organisms at times that on holding the sections to the light a thin violet line is seen running along the inner margin of the section tube, this line representing the layer of microbes (fig. E, *a*). Scattered among these specific bacilli, more especially in the upper air-passages, other kinds of microbes—micrococci and bacilli of various sizes and shapes—are also met with.

If the false membrane immediately below the superficial layer just described be now examined, the number of micro-organisms is found to be exceedingly small (fig. E, *b*); and it will be noticed that not a single bacillus is to be seen in the deeper layers of the false membrane, in the mucous membrane,

<sup>1</sup> Wagner, "Le Charbon des Poules," 'Annales de l'Institut Pasteur, Sept., 1890, p. 573.

or in the deep layers of the tissue. The whole process, therefore, as far as the bacilli are concerned, takes place on the surface of the false membrane and nowhere else.

Shortly stated, the false membrane consists of a reticulated network of fibrin, containing within its meshes a large number of wandering cells, some of which are healthy, whilst others have undergone a distinct process of degeneration. I must draw attention to the fact, however, that the wandering cells present in the membrane are of two kinds; some smaller and mono- or multi-nucleated (microphages), and others, less numerous, much larger, with a single, clear, vesicular nucleus (macrophages).

Many of the leucocytes which have penetrated between the bacilli are quite empty; but others, especially those in the layer of the false membrane close to the thick layer of bacilli previously described, contain one, two, or more diphtheritic bacilli (fig. C, *a, b*), as many as five or six micro-organisms being sometimes enclosed in one cell. Some of these intracellular bacilli appear to be healthy, staining and retaining colouring matters well; whilst other micro-organisms also contained in amœboid cells show signs of degeneration, varying from a mere difference in the power of retaining gentian-violet to complete disorganisation and destruction (see fig. E, *a, b, c, d, e, f, g*).

Many amœboid cells in the membrane, more especially those present in its superficial part, show distinct necrotic changes, many of them losing the power of retaining colouring matter. The nucleus breaks up, and does not stain at all, even with strong logwood or carmine, the surrounding protoplasm swelling up, becoming more yellow, and not unfrequently vacuolated. In later stages they show distinct signs of breaking up. Many of the degenerated cells contain bacilli in their interior, whereas others are quite empty.

Those lying between the cells in the fibrin and coagulated exudation fluid are, for the most part, quite normal to all appearance. Occasionally, though very rarely, one is met with presenting signs of degeneration similar to those found in the bacilli contained in amœboid cells. I do not think it



justifiable to argue from this fact that the inflammatory liquids present in the membrane possess a microbe-killing power, but, judging from the number of dead leucocytes present in the membrane, it is far more probable that these partially digested microbes were originally contained in a leucocyte which had died and undergone liquefaction.

Similar, but far more interesting, facts are noticed when we examine the processes taking place in chronic infectious diseases, such as tubercle and actinomycosis. Shortly stated, a nodule of tubercle or actinomycosis contains, besides the specific parasites, three kinds of amœboid cells:<sup>1</sup> 1st, small uni- or multi-nucleated cells; 2nd, large mononucleated or epithelioid cells; and 3rd, multinucleated giant-cells.

The development of giant-cells has been well studied in the *Spermophilus guttatus* by Metschnikoff.<sup>2</sup> When a *Spermophilus guttatus* is inoculated with a pure culture of the tubercle bacillus no tuberculous formations are found in any of its organs after death, but microscopical examination of the liver, spleen, and lymphatic glands shows these organs to be crammed with giant-cells, the development of which can be followed with ease.

These cells are derived from isolated epithelioid cells in the following manner:—The nucleus of an epithelioid cell assumes a star-shaped appearance, and each of the rays so produced is ultimately converted into one of the nuclei of the giant-cells. A swelling then forms at the extremity of each ray, which at first appears to be homogeneous; but later on becomes filled by a transparent mass with irregular outlines, the mass resembling a new nucleus. The chromatin now slowly divides into a peripheral and a central part, and in this way one or more nuclei are formed which are connected by a thread only with the remainder of the old star-shaped nucleus. These masses, which in the first stage are extremely irregular in form and outline, finally become detached and assume the

<sup>1</sup> I intend to discuss Baumgarten's and Weigert's views in another part of this paper.

<sup>2</sup> Metschnikoff, 'Virchow's Archiv,' vol. cxiii, 1888.

round or oval form of typical nuclei. During their formation the protoplasm of the epithelioid cell increases in size and takes the characteristic dimensions of a giant-cell. This process is not actually caused by the presence of the tubercle bacilli, for it occurs in empty cells as well as in cells containing bacilli.

Most of the bacilli contained in epithelioid cells appear to be quite healthy, but in the giant-cells degenerated micro-organisms are frequently met with. Many bacilli contained in the giant-cells are of a faint rose colour, remain unstained, or take up the blue colour only; whilst in a further stage of degeneration they become surrounded by an aureole resembling the clear space found around the pneumococcus of Friedlander. The micro-organisms become paler, stain less deeply or may even not stain at all, whilst their outlines become clearly defined. Finally they seem to disappear, but outlines of the surrounding yellowish capsule become more defined and sausage-shaped. These sausage-shaped bodies join, fuse together, lose their shape, and finally form an amber-coloured mass, which in no way resembles the bacilli from which it is derived. This process, therefore, is not a true digestive process, since the cells convert the bacilli into a solid resistant mass instead of liquefying them; it resembles rather the encapsulation of some Infusoria.

Similar forms of degeneration are to be met with in the giant-cells of tuberculous rabbits.<sup>1</sup> But although the writer has not unfrequently observed the formation of giant-cells by indirect division of the nucleus of epithelioid cells, it is quite clear that in the rabbit, guinea-pig, and man the giant-cells arise from the fusion of two or more epithelioid cells without any apparent transformation in the nucleus.

Lately fresh evidence has been accumulating to prove that the function of giant-cells formed in pathological new growths is identical with that of giant-cells found in the normal healthy body. Soudakewitch<sup>2</sup> has shown that the giant-cells of lupus in man feed on the neighbouring diseased tissue, and digest

<sup>1</sup> Metschnikoff, loc. cit., and Armand Ruffer, unpublished observations.

<sup>2</sup> Soudakewitch, 'Virchow's Archiv,' v. cxv, p. 264.

large quantities of elastic fibres. The writer<sup>1</sup> has proved that in the spleen of tuberculous animals such cells not only destroy the specific tubercle bacilli, but also devour an enormous number of red blood-corpuscles, these cells being found to be absolutely crammed with blood-pigment (figs. I, K).

I may here record an experiment which is extremely interesting, as it illustrates the development and the functions of giant-cells. Having inoculated a rabbit with 0·005 of first vaccin of charbon symptomatique, enveloped in a paper bag, I was prevented by a temporary illness from coming to the laboratory. On the sixth day the animal appeared quite well; the paper bag, together with the surrounding tissue, was taken out, hardened in absolute alcohol, and cut, and the sections stained very deeply with carmine or M. Gerrard's logwood and Grain's method. In all the sections which I examined from various situations I was unable to find a single bacillus. The cavity of the paper bag was still perfectly recognisable, although it contained the débris of the powder used, and numerous amoeboid cells. On the other hand, the outer side of the paper was already permeated by young connective tissue which was already vascularised.

I need not enter here into the description of the microphages (small mononucleated or multinucleated cells), or of the macrophages (large cells with a single clear vesicular nucleus). Suffice it to say that, contrary to the opinion of Messrs. Ballance and Sherrington, who were unable to find in their experiments any intermediate forms between the leucocytes and the plasma-cells, I maintain that every stage of development can be shown between the leucocyte and the plasma-cell. The formation of the plasma-cell in pathological effusions is identical with the formation of similar cells which I have described in normal tissues (spleen, lymphatic glands, Peyer's patch, tonsils, &c.).<sup>2</sup> Not only is their development the same, but their functions are also identical; for in the filter-paper introduced subcutaneously, as well as in the normal spleen and

<sup>1</sup> Armand Ruffer, 'British Medical Journal,' 1890.

<sup>2</sup> "On Formation of Scar Tissue," 'Journal of Physiology,' 1889, p. 558.



lymphatic glands, these cells were found to devour numbers of small lymphoid cells. Lastly, all the evidence I could gather seemed to point to the fact that the large epithelioid cells have nothing whatever to do with the development of scar tissue.

In the filter-paper we find not only these cells, but also true multinucleated giant-cells, resembling those of tubercle. They are formed by the fusion of several epithelioid cells, and it can be seen from the following facts that they have exactly the same functions as epithelioid cells. On careful examination many of them are found to contain peculiar glistening, homogeneous, irregular, yellowish bodies, which in no wise resemble anything normally found in the animal organism. These foreign bodies are nothing more than partially digested masses of filter-paper which the giant-cells have absorbed. In some cases, indeed, the cells have been fixed by the reagent at the exact moment when they were absorbing the paper fibre; one may then see one of these giant-cells almost completely surrounding the fibre, which is still partly sticking out of the cell. This new fact, together with those described by Metschnikoff, Soudakewitch, and myself, absolutely proves that the giant-cell is not a weak and diseased structure (Weigert, Koch), but an extremely active and useful body—a fighting cell.

Appearances similar to those found in tubercle are easily demonstrated in actinomycosis.

Professor Crookshank had already noticed the presence of the peculiar parasite of actinomycosis in the interior of giant-cells. More lately I have, thanks to the kindness of my friends Lingard and Sims Woodhead, obtained a large amount of material from cattle suffering from this disease; and though I must reserve details of the development of the fungi, both in animals and man, for a future occasion, I may be allowed here to draw attention to a few facts relating to the subject now under consideration as observed in actinomycosis of cattle.

A nodule of actinomycosis, which has not yet undergone caseation, consists of the central rosette formed by the parasite, and a mass of amœboid cells of various sizes, the whole nodule being surrounded by a strong capsule of connective tissue.



The amœboid cells which enter into the formation of the nodule are derived from small round mononucleated cells, which exactly resemble ordinary leucocytes. The nucleus of such a cell is round, stains deeply with hæmatoxylin or alum carmine, and shows no trace of intra-nuclear network. The delicately mottled protoplasm surrounding the nucleus varies somewhat in amount, whilst the whole cell is sometimes quite round, and occasionally slightly irregular in shape. I cannot say for certain whether these small round cells are all derived from emigrating leucocytes, or whether some are not derived from pre-existing connective-tissue cells. Both modes of origin are probably the rule, for at the periphery of the new growth—in places where no parasites can be discovered—the connective-tissue cells are often in a state of active proliferation. The blood-vessels, their walls, and their immediate neighbourhood, on the other hand, are frequently crammed with leucocytes.

It is quite easy to trace the development of epithelioid and giant cells from leucocytes in the nodule of actinomycosis. The nucleus of some of the small lymphocytes is occasionally composed of a dark outer border, and a clear, but somewhat swollen, centre (fig. M, *a*). In others the centre of the nucleus becomes clearer, and the chromatin almost entirely disappears in places, thus leaving a fine intra-nuclear network possessing one or more nucleoli (fig. M, *b*). Owing to the gradual disappearance of the chromatin in the centre and periphery, the nucleus becomes clear and bladder-like, and possesses a beautiful intra-nuclear network (fig. M, *c*); but the protoplasm in this stage has as yet undergone no change, and the whole cell may not be larger than an ordinary lymphocyte.

The nucleus now slowly becomes larger, the protoplasm increases enormously in size, becomes coarser and vacuolated, with extremely irregular contours, and the cell finally presents the appearance characteristic of an epithelioid cell (fig. M, *d*, *e*). The nucleus now gradually changes its place and approaches one of the poles of the cell.

Just as in the healthy lymphoid tissues of animals the larger amœboid cells or macrophages take into their interior

and destroy the smaller ones, so do the macrophages met with in actinomycosis destroy the microphages. It is easy to find such macrophages containing one, two, or as many as five or six microphages in various stages of degeneration (figs. L, m, and M, *h, j, k, l*; also fig. H). Several epithelioid cells may then coalesce and form a giant-cell (fig. H, *a, b, c, d, e*); but I have never seen division of nuclei as in the epithelioid cells of *Spermophilus guttatus*.

Near the growing edge of the tumour each rosette is surrounded by a layer of epithelioid and giant cells, forming a regular palisade around the nodule. The nuclei of such cells are always placed at the pole situated away from the parasite.

The epithelioid cells in other places penetrate into the rosette of actinomycosis and take into their interior huge bunches of these parasites (fig. F), which then undergo degeneration in the interior of these cells. The giant-cells likewise often contain masses of such parasites (fig. G). At the growing edge of the tumour, however, giant-cells are few, and, as might be expected, there is no formation of new connective tissue.

In the parts of the tumour which are older, and therefore harder and more fibrous, the appearances are very different. The periphery of each nodule is surrounded by a dense capsule of fibrous tissue. The epithelioid cells, however, take no part in the formation of this fibrous tissue, for in no case could I see appearances justifying such an assumption. On the contrary, the connective tissue is derived from small cells possessing a hard, darkly staining single nucleus. These cells gradually elongate, the nucleus becomes oval and clearer at the same time, until typical long spindle-shaped connective-tissue cells are produced (fig. J).

The appearances of the disease in a fibrous part of the tumour are very different from those seen near the growing edge. The older parts generally contain a large number of epithelioid cells and giant-cells, holding in their interior bunches of actinomycosis, which often show signs of degeneration. Instead of staining of a dark purple colour with gentian-violet the parasites do not stain at all, but become con-

verted into a homogeneous, highly-refracting mass, consisting of sheaths of the dead parasites. At the same time, however, the giant-cells and epithelioid cells suffer in the fight, their nuclei become granular, the protoplasm liquefies, and so finally parts of the tumour consist of actinomycosis—few of which show any signs of life—and masses of dead amœboid cells. In other parts everything is dead, and nothing is left but the dead bodies of the parasites and pus-cells. Strangely enough when this is the case, the lymphoid cells in the surrounding parts do not appear to make the slightest attempt to take the dead particles into their interior, but neglect them in favour of the living leucocytes and fungi in their neighbourhood—a proof, if any were wanting, that phagocytes do not eat up everything they come across, but exert a distinct choice.

In actinomycosis, therefore, as in tubercle, the amœboid cells which enter into the formation of the “pathological granuloma” are fighting cells which actively destroy the invading parasites.

Although many facts have accumulated to give us some idea as to what happens when micro-organisms are introduced into the subcutaneous tissue, our knowledge of the processes following on their forcible introduction into the blood is still extremely meagre.

According to Wyssokowitch<sup>1</sup> micro-organisms are not destroyed in the blood, but remain for a certain length of time in the organs, more particularly in the lymphatic glands, the medulla of bones, and the spleen. Wyssokowitch found them in the endothelial cells of blood-vessels. Micro-organisms, according to the same author, are not eliminated by the urine, unless through some lesion of the kidney (diapedesis, rupture of vessel, abscess, infarctus, &c.); Berlioz<sup>2</sup> has arrived at the same conclusion. That this, however, is not an absolute rule has been proved by Charrin<sup>3</sup> and myself.<sup>4</sup>

<sup>1</sup> Wyssokowitch, ‘Zeitschrift f. Hygiene,’ 1886, T. i, Heft 1, p. 45.

<sup>2</sup> Berlioz, ‘Thèse de Paris,’ 1888.

<sup>3</sup> Charrin, ‘La Maladie Pyocyane.’

<sup>4</sup> Armand Ruffer, ‘Experimental Investigation into the Nature of the Disease produced by the Inoculation of the *Bacillus pyocyaneus*.’



In a series of experiments Wyssokowitch injected into the veins of animals—1st, simple saprophytes; 2nd, bacilli pathogenic for certain animals, but harmless for the animals used; 3rd, pathogenic micro-organisms; 4th, micro-organisms which become pathogenic only when injected in large quantities. Examining the blood of animals by cultivating it on gelatine plates, he found that non-pathogenic micro-organisms disappeared with the greatest rapidity, so that the blood contained none after three hours. The micro-organisms belonging to the second class disappeared more slowly, i. e. in twenty-four hours; whilst pathogenic micro-organisms, e. g. anthrax, diminish in number at first, so that none could be found after four hours, but then multiplied rapidly, so that the blood twenty-four hours after inoculation contained an incredible number. The bacilli of anthrax pass into the urine only a few hours before death when the urine contains blood; other micro-organisms do not pass in the urine unless some lesion of the kidney is present.

Non-pathogenic micro-organisms which form spores disappear from the blood with exceeding slowness. Thus the spores of the *Bacillus subtilis*, when the latter micro-organism is injected into the blood, are met with in the liver and spleen two or three months after the injection, even when the animals have remained in perfect health.

Wyssokowitch has found the micro-organisms of suppuration (*Staphylococcus aureus* and *S. albus*) in the cells of the milk of women suffering from puerperal fever. According to Malvoz,<sup>1</sup> the passing of bacilli from the maternal to the foetal placenta only takes place when slight vascular ruptures are present.

Clinical facts also show that repeated examinations of the blood in the large majority of infective diseases does not reveal the presence of micro-organisms in that fluid. In typhoid fever, for instance, the bacilli are never met with in the blood. (For an excellent résumé of this question see J. Gasser, 'Arch.

<sup>1</sup> Malvoz, 'Annales de l'Institut Pasteur,' 1888, p. 121.



de Médecine Expérimentale et d'Anatomie Pathologique,' vol. iii, No. 1, 1891, p. 119.)

Such competent observers as Pfuhl,<sup>1</sup> Merke,<sup>2</sup> Leitz, and Lucatello,<sup>3</sup> failed to find any micro-organisms in the blood of patients during the third and fourth week of typhoid fever. Neuhaus<sup>4</sup> examined the blood in nine cases of the same disease, by inoculating it on gelatine tubes; all the tubes remained sterile, with the exception of six, which had been inoculated with the blood coming from the spots of six different patients. Rüttimeyer<sup>5</sup> came to similar results, and hence we may conclude that in this disease the blood contains the specific bacilli in an intermittent manner only, although the spleen<sup>6</sup> always contains them. These clinical results agree exactly with the experimental data; for it has been found<sup>7</sup> that if a pure culture of typhoid bacilli be injected into the veins of a rabbit, the animal killed after eighteen hours, and particles of organs, &c., sown on gelatine plates, the number of colonies varies with the organs used.

|                      |   |   |   |   |               |
|----------------------|---|---|---|---|---------------|
| Thus the spleen gave | . | . | . | . | 242 colonies. |
| Medulla ossium       | . | . | . | . | 200 „         |
| Liver                | . | . | . | . | 12 „          |
| Heart                | . | . | . | . | nil.          |

The bacillus of tubercle in man at least has only once been met with in the blood,<sup>8</sup> and yet it is undoubtedly carried about by this fluid. Thus meningeal tubercles are often closely applied to a small artery which is obliterated by fibrin. In the tunica intima of vessels of tubercular meninges<sup>9</sup> are found a large number of bacilli, small cells and giant-cells; and all

<sup>1</sup> 'Deutsche Militärärzte Zeitschrift,' 1886, p. 23.

<sup>2</sup> 'Munich Med. Wochenschrift,' 1880, p. 491.

<sup>3</sup> 'Bull. d'Académ. de Genova,' ii, 3, 1887.

<sup>4</sup> 'Berlin Klin. Wochenschrift,' 1886, p. 389.

<sup>5</sup> 'Correspondenzblatt f. Schweiz Aerzte,' p. 397, 1887.

<sup>6</sup> Chantemesse and Widal, 'Arch. de Physiologie,' March, 1887.

<sup>7</sup> Wyssokowitch, loc. cit.

<sup>8</sup> Weichselbaum, 'Wiener Med. Wochenschr.,' 1884, No. 12.

<sup>9</sup> Cornil, "Contribution à l'étude de la Tuberculose," 'Journ. de l'Anatomie,' 1880.

these may be met with even in the clot obstructing the vessel. In general miliary tuberculosis, moreover, tubercular granulations of the intima of pulmonary veins, of the right endocardium, or inferior vena cava, are generally found. In patients suffering from miliary tuberculosis<sup>1</sup> bacilli have been met with post mortem in the clots of large vessels. In experiments in which the *Bacillus pyocyaneus* had been injected into the veins, it was not unfrequently found that the blood contained no micro-organisms,<sup>2</sup> when the liver, kidneys, lungs, and even urine, were swarming with them. The blood, therefore, is not their usual habitat; but micro-organisms, like inert powders, injected into the blood, are arrested wherever the circulation is slowed by any cause; that is, in the liver, spleen, kidney, marrow of bone, lymphatic glands, &c. When injected in large quantities, the bacilli may produce lesions simply by their numbers. Thus most beautiful and typical infarcts can be produced by injecting large quantities of a culture of *Bacillus pyocyaneus* into an animal which has been rendered artificially immune.<sup>3</sup> One thing is certain, namely, that the activity of the lymphoid cells contained in the blood and other tissues varies exceedingly, according as the animal possesses some degree of immunity or not. Thus, according to Metschnikoff, when virulent anthrax is injected into a rabbit, the bacilli in the blood are extremely numerous, and lie free in the fluid. The same observer rendered animals immune against anthrax by Pasteur's method; he then inoculated virulent anthrax, and examined the blood at varying intervals after the inoculation. Sixteen hours after the inoculation some bacilli were still free in the fluid, while many were contained in white corpuscles. Twenty-two hours after the inoculation all the bacilli were contained in lymphoid cells; or if by any chance a number of them were found floating free in the liquid, they were surrounded by a mass of white corpuscles. Three days afterwards all the bacilli had disappeared.

<sup>1</sup> Weigert, "Zur Lehre von der Tuberculose," 'Virchow's Archiv,' 1882.

<sup>2</sup> Charrin and Armand Ruffer, loc. cit.

<sup>3</sup> Charrin and Armand Ruffer, C. R., 'De la Société de l'Anatomie,' 1889.

Hesse has arrived at similar results by injecting virulent anthrax into the veins of animals, such as frogs and dogs, which are naturally immune against anthrax.

In tuberculosis, also, giant-cells are met with in all tubercular tissues;<sup>1</sup> but one may say that whenever the bacilli of tubercle invade the tissues through the blood, giant-cells are met with in the blood-vessels of these regions. When a pure culture of virulent tubercle bacilli is injected into the lateral vein of the ear of a rabbit, the bacilli, becoming arrested in the veins and capillary vessels of the liver and spleen, give rise to small fibrinous coagula, in which they multiply up to the fifth or seventh day. After a week the cells of the spleen and the leucocytes proliferate actively in the vessels, and the colonies of bacilli become surrounded by migrating cells, which develop into epithelioid and giant cells.

Up to the present time I have only stated the microscopical facts which may be observed under the microscope. I have not attempted to explain why the cells migrate or not out of the vessels. This question will be fully discussed in the next part of the paper, when I intend to speak of the influence exerted on amœboid cells and on the other anatomical elements of the tissues by the chemical poisons secreted by micro-organisms.

NOTE.—In a lecture delivered before the Royal Institution on February 20th, 1891, my friend Dr. E. Klein, F.R.S., vehemently attacked the whole theory of immunity being due to the action of phagocytes. I do not intend to break through the plan of this work in order to answer Dr. Klein's objections, as the facts on which he bases his theories will be fully discussed later on; but I take this opportunity of saying at once that I fully maintain the truth of every one of the observations which I have made, and every new fact which I have observed lately confirms me in my opinion.

<sup>1</sup> Cornil and Babes, 'Les Bactéries,' vol. ii, p. 92.

## DESCRIPTION OF PLATES XXXII &amp; XXXIII,

Illustrating Dr. M. Armand Ruffer's paper on "Immunity against Microbes."

FIG. A.—Wall of abscess, following an inoculation of the weak virus of quarter-evil into a guinea-pig. Alum-carminé and gentian-violet. Vérick, oc. I,  $\times \frac{1}{12}$ th. *a, b, c, d* point to bacilli in various stages of degeneration.

FIG. B.—From the same section as the preceding. Vérick, oc. III,  $\times \frac{1}{13}$ th. For letters see text.

FIG. C.—Sections through superficial part of the diphtheritic membrane in man. Gentian-violet and alum-carminé. Vérick, oc. I,  $\times \frac{1}{13}$ th. *a* and *b* point to cells containing bacilli in their interior.

FIG. D.—Section of diphtheritic membrane under a low power. *a* represents the layer of bacilli; *b* the membrane underneath.

FIG. E.—From the same section as preceding. Gentian-violet and alum-carminé. Vérick, oc. III,  $\times \frac{1}{12}$ th. Leucocytes of diphtheritic membrane containing bacilli in various stages of degeneration.

FIG. F.—Epithelioid cells containing clubs (actinomycosis). Logwood and fuchsine staining. Vérick, oc. III,  $\times \frac{1}{12}$ th.

No. 1.—*n*. Nucleus of cell. *a, b, c*. Clubs in various stages of degeneration.

No. 2.—*n*. Nucleus. *a, b, c*. Clubs of actinomycosis in various stages of degeneration. *l*. Débris of lymphocyte.

Nos. 3, 4, 5.—*n*. Nuclei. *a, b, c*. Débris of clubs and vacuole.

No. 6.—*n*. Nucleus. *a, b, c, d, e*. Débris of clubs. *l*. Débris of lymphocytes.

FIG. G.—Giant-cell, containing—*a*. Degenerated bunch of actinomycosis.

FIG. H.—Giant-cells in actinomycosis.

FIG. I.—Epithelioid cells from tubercular guinea-pig's spleen. Logwood staining. Vérick, oc. I,  $\times \frac{1}{12}$ th. *n*. Nucleus. *a*. Blood-pigment.

FIG. J.—Development of connective tissue at the periphery of a nodule of actinomycosis.

FIG. K.—Giant-cell from tubercular guinea-pig's spleen, containing blood-pigment.

FIG. L.—Epithelioid cells from nodule of actinomycosis, containing in their interior leucocytes in various stages of degeneration.

FIG. M.—Development of epithelioid cells from lymphocytes in nodule of actinomycosis. Logwood staining. Vérick, oc. I,  $\times \frac{1}{12}$ th.



**The Formation and Fate of the Primitive  
Streak, with Observations on the Archen-  
teron and Germinal Layers of *Rana tem-  
poraria*.**

By

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and

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With Plates XXXIV and XXXV.

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EVEN a superficial perusal of the literature of the development of the Amphibian ovum is sufficient to acquaint the inquirer with numerous contradictory statements concerning points of weighty morphological importance. Not only are there differences of opinion as to the interpretation of some of the developmental features which are generally allowed to be readily recognisable, but there are also assertions, made by different observers, concerning merely the structural arrangement and the fate of various portions of the Amphibian ovum which are absolutely irreconcilable with each other. The confusion is added to by a loose application of terms describing changes and areas, so that it becomes difficult to decide the exact relation that the Amphibian ovum bears to the ovum of other Vertebrates.

In view of the chaos which already exists we would not readily enter upon any further discussion of Amphibian

development, were it not that we think the results of our recent observations on the development of the Anura tend to simplify the problem we have been studying by throwing light upon several interesting developmental phenomena. For these reasons alone are we induced to publish the results of a research which tend to conclusions different from those of our predecessors, whose experience is, in most cases, much more extensive than our own.

At the outset it may, with advantage, be stated that our observations were directed principally to the mode of formation of the archenteron and blastopore, the fate of the blastopore, and the formation and fate of the primitive streak; but incidentally we have dealt with the separation of the germinal layers and the relation of the mesoblast to the chorda and hypoblast.

We worked independently of each other. One of us was led on to the investigation by the contradictions between the current statements regarding and the obvious facts disclosed by the developing anural ovum; whilst the other approached the subject with the conviction, based upon theoretical grounds, that either the descriptions of certain phases of Amphibian development were incorrect, or that there were very peculiar and significant differences between the Amphibian and other Vertebrate ova. Until our conclusions had been arrived at neither of us was aware that the other was engaged upon the subject. Consequently our observations were made and our conclusions formed independently of each other.

Methods.—Living and hardened ova were examined, but we relied chiefly upon sections cut in the three usual planes, horizontally, sagittally, and transversely; and of these the horizontal have proved in some respects the most useful and instructive, more especially in the observations upon the fate of the primitive streak.

The ova were hardened either in Perenyi's fluid or in Kleinenberg's picro-sulphuric acid solution. The former fluid was most useful in the younger stages. After the hardening was completed the ova intended for sections were embedded in paraffin in the usual manner.

None of the ova were stained, for our experience has been that the solutions through which the ova are passed, before the staining is complete, alters the relations of the cells, and that the staining hinders rather than facilitates the microscopical examination of sections of the younger stages.

We obtained several surface views which were useful for comparison with the results of previous observers; of these the one represented in fig. 11 was drawn from a living embryo.

Fig. 23 was drawn partly from a surface view of an ovum hardened in Kleinenberg's fluid, and partly from a model constructed by putting together, in order of sequence, pieces of cardboard cut to correspond with camera drawings of a complete series of transverse sections. Our work was greatly facilitated by Professor Marshall, who very kindly placed at our disposal a large series of sections, and to him also our thanks are due for much kind advice.

Before proceeding to the description of our own observations, we must refer shortly to previous records, for by this course alone shall we be able to indicate clearly the differences between our results and those of the observers who have preceded us, and at the same time we shall obtain an opportunity of defining some of the terms that we shall be obliged to use in our description.

Turning, therefore, in the first place to the consideration of the formation of the archenteron and the blastopore (but leaving aside for the present the concrescence theory of His [22 and 23], so far as it concerns the formation of this cavity), we find that upon this, as upon most other important points, there is a distinct difference of opinion between the previous observers. It is stated by some that after the formation of the segmentation cavity, and when the ovum is only partially covered by the pigmented epiblast-cells, the archenteron is formed by an invagination, which "first commences by an inflection of the epiblast-cells for a small arc on the equatorial line which marks the junction between the epiblastic cells and the yolk-cells" (1, p. 102).

This preliminary invagination is also described by Perenyi

in Bombinator (40), O. Schultze in *Rana fusca* (45), and by Scott and Osborn in the Newt (48).

Whilst it is proceeding the enclosure of the yolk has been rapidly taking place. "It is effected by the epiblast growing over the yolk at all points of its circumference" (1, p. 102). We have in this latter statement of Balfour's a very fair summary of the general opinion that the epiblast and yolk are distinct parts of the ovum; indeed, Balfour speaks of the yolk-cells as "these large cells which are part of the primitive hypoblast" (1, p. 101), with which, in our opinion, they cannot fairly be compared; and we shall speak of them in the sequel merely as yolk-cells, though probably the more correct term would be germ segments. It is not necessary, however, to enter into a discussion of the mode of extension of the epiblast at the present moment, and we can proceed, therefore, to a point which is allowed by all, namely, that the superficial extension of the epiblast terminates at the margin of a large circular opening, called the blastopore or the anus of Rusconi, at the margin of which the surface epiblast becomes continuous with the cells lining the archenteric cavity, which, on account of their position, are spoken of as hypoblast, although those situated in the dorsal wall of the cavity are said to be invaginated epiblast in the Newt (48), or partly epiblast and partly differentiated yolk-cells in the Anura (1, p. 102), whilst the lateral walls and the floor of the archenteron are formed by modified yolk-cells.

Houssay (26) and Moquin-Tandon (38) are, so far as we are aware, the only authors who have combated this supposititious mode of formation of the archenteron. Houssay examined the Axolotl and Moquin-Tandon several Anura. They both state that the archenteron is formed by splitting amidst the yolk-cells, from which it follows that both in the Urodela and Anura there is no invagination of epiblast into the yolk, and that the archenteron is surrounded by modified yolk-cells which eventually form a distinct hypoblastic layer, which is continuous with the epiblast at the margin of the blastopore.

The blastopore is unanimously defined in Amphibians as an



opening, round the margins of which the epiblast and hypoblast are continuous, and which forms the passage of communication between the archenteron and the exterior. But concerning the formation and surroundings of the archenteron there are two opposed opinions, which are—

1. That the archenteron of the Amphibia is a cavity, formed as in *Amphioxus* by invagination, and is lined partly by modified yolk-cells and partly by invaginated epiblast.

2. That the archenteron is formed in situ by splitting amongst the yolk-cells, and that it is entirely surrounded by modified yolk-cells.

To these opinions it will be necessary to return, but in the meantime we pass to a consideration of the various accounts of the fate of the Amphibian blastopore.

This opening, after remaining for a time, is, according to Balfour's account, decreased in size by the approximation of its lips until it forms "a narrow passage, on the dorsal side of which the neural tube opens. . . . The external opening of this passage gradually becomes obliterated, and the passage is left as a narrow diverticulum leading from the hind end of the mesenteron into the neural canal (1, p. 108). It forms the post-anal gut, and gradually narrows and finally atrophies." This account is in the main conformable with Goette's first description of *Bombinator*: "Die verengte sich vorherrschend von beiden Seiten her, sodass sie Spaltartig wurde und ihr Längsdurchmesser in der Medianebene des sich entwickelenden Embryonalkörper lag" (14, p. 132). And, further, Balfour (1, p. 108) states that the anus is formed in *Rana temporaria* at an earlier period than in *Bombinator*, as described by Goette, but in the same manner, that is, by the fusion of a diverticulum from the mesenteron with a cutaneous invagination. Almost the same change takes place in *Rana fusca* (46); Spencer, however, as a result of his study of *Rana temporaria*, came to the conclusion that the blastopore was transformed into the permanent anus (52); and Alice Johnson and Lilian Sheldon (28) arrived at a similar conclusion concerning the blastopore of *Triton cristatus*, which,

according to the account of Scott and Osborn (48), is enclosed by the neural folds.

Durham (9) describes a neurenteric canal and a blastopore present at the same time in *Rana*; and Sidebotham (51) asserts that the neural folds do not enclose the blastopore, which is closed subsequently to the meeting of the neural folds, and that the anus is derived from an independent proctodæal invagination.

Morgan's (39) interesting observations tend to a solution of the difficulty caused by the preceding contradictions, inasmuch as they show that in *Amblystoma punctatum* the mesial portions of the lateral lips of the blastopore are first approximated, the blastopore thus becoming hour-glass-shaped, and then fused, so that the previously single opening is divided into two; the anterior of the two is enclosed by the medullary folds, and becomes the neurenteric canal; the posterior remains as the anal orifice. Goette (15) has lately described a somewhat similar condition in *Bombinator*.

In *Rana helecina*, according to Morgan (39), there is behind the blastopore a groove communicating posteriorly in a dark spot, which is a later formation than the blastopore. In describing a very similar groove in *Bufo lentiginosus* he states that it is separated from the archenteron by three embryonic layers, a point of importance in any comparison of this region with the primitive streak of other Vertebrates.

Erlanger's (10A) observations upon the *Anura* have led him to the conclusion that the blastopore closes both from before backwards and from behind forwards. The closure from before backwards is associated with the formation of the primitive streak, which lies in front of the neurenteric canal. The closure from behind forwards gives rise, in the first instance, to a fused mass of cells, which rapidly differentiates into layers, after which the anus forms in the situation previously occupied by the posterior portion of the blastopore. Therefore, so far only as the statements of the authors to whom we have referred are concerned, the blastopore of Amphibians may either—

- Become (1) gradually constricted, being transformed into a narrow canal, which for a time unites neural and alimentary cavities, but eventually disappears—Balfour (1), Schultze (46), Scott and Osborn (48);
- Or (2) it is transformed into the anus—Alice Johnson (27), Spencer (52);
- Or (3) it is not enclosed in the neural folds, it does not form the anus, but gradually disappears—Sidebotham (51);
- Or (4) the anterior part becomes the neurenteric canal and the posterior part the anus—Morgan (39), Schwarz (47), Goette (15);
- Or (5) the anterior portion becomes the primitive streak, the middle portion the neurenteric canal, and the posterior part, after being closed, is again reopened in a small portion of its extent as the anus—Erlanger (10A).

But this summary does not include all the fates which are allotted to the blastoporic opening, for its history has been intimately connected with that of the primitive streak and the axial line of the embryo by the researches of His (22 and 23) and Rauber (42), whose observations resulted in the "conrescence theory" of Vertebrate development, which Minot (36) has striven to support in his recent papers. It is necessary to consider this theory, as the results of our observations bear upon it; but before referring further to it we must draw attention for a moment to the structure of the lips of the blastopore. It is almost universally allowed that in all Vertebrates, with the exception of *Amphioxus*, there is in this region a fusion of all three layers of the germ, and it follows as a consequence, if the lateral borders of the blastopore are approximated and united, that there should result a mesial axial rod of fused tissue, which cannot in the first instance be resolved into distinct layers. Such a fusion of the blastopore lip was long ago shown to occur in *Elasmobranchii* by Balfour (1, p. 51), who compared the line of fusion of the lips of the *Elasmobranch* blastopore to that portion of the blastodermic area of the Avian ovum to which the term primitive streak



had been applied. "The primitive streak represents the linear streak connecting the Elasmobranch embryo with the edge of the blastoderm after it has become removed from its previous peripheral position, as well as the true neurenteric part of the Elasmobranch blastopore" (1, p. 238). . . . "That it is in later stages not continued to the edge of the blastoderm, as in Elasmobranchii, is due to its being a rudimentary organ" (1, p. 240). In Balfour's opinion, therefore, the primitive streak represents a portion of the blastoporic opening situated posterior to the neurenteric canal, and in conformity with his statement that the anus of Rusconi in the frog is the whole of the blastopore, which becomes gradually contracted, he does not describe a primitive streak in Amphibia. His conclusions, however, are not in accord with those of the upholders of the concrescence theory, who believe that the axial portion of the embryo is formed by the fusion of the lips of an elongated blastopore. The fusion takes place from before backwards, and results in the formation of an axial area, which lies in front of the remains of the blastopore. This area is termed the primitive streak.

If we examine the anus of Rusconi of Amphibia in the light of Balfour's conclusions concerning the blastopore, and then in that of the concrescence theory, we are forced to believe in the first case that either there is no primitive streak in Amphibians, or that it lies somewhere posterior to the neurenteric canal; and, further, that it would be completely represented by the fusion of the middle part of the lateral lips of the blastopore in *Amblystoma punctatum* (39), and in *Bombinator* according to Goette's description; or, in the second case, that the anus of Rusconi represents the posterior part of the blastopore, and that the primitive streak is situated in front of it.

Schwarz (47) and Goette (15) compare the "Prostoma-schluss" which lies between the neurenteric canal and anus in *Bombinator* to the primitive streak of the higher Vertebrates.

Morgan (39), Alice Johnson (27), and Schultze (45) mention a primitive streak in front of the blastopore. Morgan gives no



description either of the formation or structure of the streak. Alice Johnson (27) describes a fusion of the germinal layers in the region in front of the blastopore which she calls the primitive streak, but she does not say whether the fusion is primary or secondary. There is, however, no doubt about the formation of the area called primitive streak by Schultze in his description of *Rana fusca*, for he describes the process in the following words:—"Während auf den vorhergehender Entwicklungsstadien dorsal und median das äussere Keimblatt durch einen Spaltraum von dem mittleren Blatt getrennt war, und beide Blätter nur an der ringförmigen Zone der Blastoporuslippe in einander übergingen, bildet sich gegen Ende der Gastrulation, von der Mitte der dorsalen Urmundlippe aus, eine nach dem Kopfe hin vorwärts schreitende lineare Verwachsung, des Ausseren und mittleren Blattes aus" . . . . "diese lineare Verwachsung, in welcher wie in dem Primitivstreifen des Höheren Wirbelthiere Ektoblast und Mesoblast zusammenhängen; von der dorsalen Urmundlippen sich nach dem Kopfe hin allmählich ausdehnt, und wächst also der Primitivstreifen auch bei den Amphibien von hinten nach vorn" (45, p. 330).

This statement of Schultze's tends to increase the difficulty of the situation, for it agrees neither with Balfour's nor with the concrescence theory of the primitive streak. In opposition to Balfour's theory Schultze places the primitive streak in front of the neurenteric canal, and he says that the streak is formed from behind forwards, a statement incompatible with the concrescence theory, which necessitates that "the entodermal canal and primitive streak begin at the edge of the blastoderm and grow at their posterior end away from the segmentation cavity, and at the same rate the blastoderm over-spreads the yolk" (36, p. 511).

Neither do Erlanger's observations (10A) tend to simplify the problem. He associates the primitive streak with the antero-posterior closure of the dorsal portion of the blastopore, but according to his figures the primitive streak ultimately exceeds in length the diameter of the primitive blastopore. He figures no sections to show the structure of the streak, and we

are inclined to believe that he has mistaken the neural furrow for the primitive streak. But if his conclusions are correct, they support the concrescence theory only in part, in so far as the fusion from before backwards of a portion of the blastopore is concerned; whilst the fusion of the lip of the ventral portion of the blastopore, which he describes as taking place from behind forward, is at variance with the concrescence theory.

Nevertheless, to the summary which has already been given of the fate of the blastopore in Amphibians (p. 457), we must add the further possibility that, according to the concrescence theory, the posterior part of it only is to be recognised in the anus of Rusconi, the lateral lips of the anterior part having undergone a "retrogressive fusion" similar to that described by His in bony fishes (22), and that this fusion is continued until the complete obliteration of the blastopore results.

From the statements noted in the preceding survey of the literature of the subject we may deduce the following summary of the presence or absence and the mode of formation of the primitive streak in Amphibia:

1. There is no mention of a true primitive streak—Balfour (1).

2. There is a primitive streak situated in front of the anus of Rusconi—Schultze (45), Minot (36), Alice Johnson (27), Erlanger (10A).

3. The fused lips of the blastopore between the neurenteric canal and the anus represent the primitive streak—Schwarz (47).

The primitive streak is formed—

1. By retrogressive fusion of the lips of the blastopore (concrescence theory).

2. By fusion of the lips of the blastopore between the neurenteric canal and anus—Schwarz (47).

3. By fusion of the epiblast and mesoblast from behind forwards in front of the anus of Rusconi—Schultze (45).

Before concluding this short survey of previous records we wish to summarise the statements, some of which have been

already referred to concerning the formation of the anus in Amphibians.

1. It is a new formation in the region below the blastopore—Balfour (1), Sidebotham (51).

2. It is the blastopore—Sedgwick (49), Alice Johnson (27), Spencer (52).

3. It is the posterior portion of the blastopore—Morgan (*Amblystoma punctatum*) (39), Schwarz (47), Goette (15).

4. It is a secondary opening in the situation of the posterior part of the primitive blastopore—Erlanger (10A).

#### The Enclosure of the Yolk by Epiblast and the Formation of the Archenteron.

At the period generally spoken of as the end of the segmentation the anural ovum is a sphere which contains an excentrically situated segmentation cavity.

The segmentation cavity has a roof which ultimately becomes the anterior wall of the gastrula; for the anus, which marks the posterior end of the embryo, appears at the opposite pole of the ovum, that is in the floor of the segmentation cavity.

The roof of the cavity is formed by two or three rows of comparatively small pigment-bearing cells, and the floor by a mass of ill-defined nutriment-laden cells which collectively form the yolk. At the margin of the segmentation cavity the two walls merge into each other by a series of insensible gradations, so that it is impossible to say where one ends and the other begins. Nevertheless it is certain that the cells of the roof of the segmentation cavity are epiblast, for they ultimately form a portion of the external covering of the embryo; but it is incorrect to speak of the opposite wall, the yolk-cells, as modified hypoblast (1, p. 103), unless it is allowed that epiblast may be formed from modified hypoblastic cells. For during the formation of the blastopore the epiblast does not grow over the yolk-cells, enclosing them by a process of epibolic invagination. If it did, it would be possible to recognise on section a line along which the epiblast terminated. No one has described or figured such a line of limitation except dia-



grammatically; and a careful examination of thin sections of anural ova, in the stages preceding the completion of the circular blastopore, shows clearly that the descriptions given of the gradual extension of the epiblast over the yolk represent theory rather than fact.

The changes observable are, firstly, that the cells of the superficial layer of the yolk gradually become more distinctly pigmented; and, secondly, that they thereafter divide into smaller and more distinct segments, which arrange themselves into two layers, a superficial layer of somewhat cubical deeply pigmented cells, and a deeper layer of less pigmented and more rounded cells, irregularly arranged into two or more rows: the latter are separated by a distinct space from the subjacent remainder of the yolk-cells. This transformation and rearrangement proceeds backwards towards the blastopore, stopping about .352 mm. from that aperture, first on its dorsal and then on its lateral and ventral borders, where an area of fusion remains, which is for convenience divided into a dorsal, a ventral, and two lateral lips or borders.

We conclude, therefore—(1) That the segmentation of the anural ovum does not result in the formation of a vesicle, the roof of which is epiblast and the floor modified hypoblast, but that at the end of the segmentation the primary layers of the ovum are only partially formed. The roof of the segmentation cavity is epiblast, but the floor or yolk is not modified hypoblast. It consists of indifferentiated germ-cells, the true characters of which are not at first recognisable.

(2) That the yolk is not enclosed by the gradual extension over it of a previously differentiated epiblast, but that the superficial layer of yolk-cells becomes gradually differentiated into the two layers of epiblast, leaving a remainder of the yolk. This consists of hypoblast and mesoblast, which are not at first separated from each other.

The formation of epiblast from yolk-cells does not take place over the whole surface of the yolk, for at the posterior pole of the ovum there is a circular patch of yolk-cells from which no epiblast is formed. The cells in question form the



yolk-plug, and when the epiblast formation has arrived from all sides at their margin, the circular blastopore or anus of Rusconi is first definitely established.

During the period of differentiation of the epiblast the archenteron has also been forming. The first indication of this cavity is the appearance of a curved area of pigmentation (fig. 1 *a* and fig. 2 *AR*) of semilunar outline amidst the yolk-cells at the posterior pole of the ovum above the equator. The convexity of the pigmented area is directed forwards amidst the yolk-cells, towards the segmentation cavity. The angles are turned ventro-laterally. The centre of the concavity of the semilunar area corresponds to the dorsal lip of the blastopore. As the convexity of the area extends forwards towards the segmentation cavity, its lateral angles travel ventrally along the lines of the lateral lip of the blastopore until they meet in the situation of the ventral lip of that opening, which is thus marked out by a line of pigmentation that indicates the situation of the future cavity.

The pigmented area is produced and extended by the deposit of pigment in the adjacent margins of a double row of yolk-cells in the manner shown in figs. 1 and 2, which are drawings of sections of the advancing anterior extremity of the pigmented area of a much more developed ovum. The pigment is deposited in the protoplasm of the double row of cells which, eventually, will form the boundary wall of the archenteron, and it also radiates from this area along the adjacent margins of the cells of each row.

It is to be understood, however, that directly after the pigmented area is first formed, and as it extends forwards and ventrally, a slit-like space appears in the middle of its posterior portion. This space first limits the dorsal lip of the blastopore, and then extends forwards and ventrally, following the deposit of pigment, and separating the two rows of marginally pigmented cells from each other. It is the archenteric space. As its lateral angles extend ventrally along the line of pigmentation they define the lateral lip of the blastopore; their union ventrally gives rise to the ventral lip, and

at the same time defines the posterior limit of the ventral wall of the archenteron (fig. 3).

A diagrammatic representation of a sagittal mesial section of the ovum at the period of completion of the blastoporic lip is given in fig. 4, from which it will be readily seen that, at this period, the ventral wall of the archenteron extends from the point *a* to the point *x*. In a portion of its extent the floor of the slit-like cavity lies parallel to the superficial surface of the ovum, but posteriorly it is projected outwards into a deficiency in the external wall (the blastopore), forming in this region the yolk-plug or bouchon d'Ecker. At the same stage the dorsal wall of the archenteron is not co-extensive with the ventral. It extends only from the point *a* to the point *b*, which marks the dorsal lip of the blastopore. From the dorsal to the ventral lip of the blastopore the wall of the archenteron is deficient. It will be shown afterwards how this portion of the wall, which we shall call the posterior wall of the archenteron, is completed.

At present we more particularly desire to call attention to the fact that the point *x*, which at this period marks the posterior limit of the ventral wall of the archenteron, remains fixed throughout the later periods, that eventually it is situated just in front of the anal orifice, and that there is no extension ventrally of the archenteron beyond it, such as that described by Balfour, who, speaking of the gradually narrowing blastopore, says: "At its front border, on the ventral side, there may be seen a slight ventrally directed diverticulum of the alimentary tract, which first becomes visible at a somewhat earlier stage" (1, p. 108). On the contrary, the ventral wall of the archenteron is only increased in front of the ventral lip of the blastopore by the forward extension of the anterior end of the cavity and the growth of the ovum, and is completed immediately in front of the ventral lip, so far as we have been able to ascertain, by the gradual withdrawal of the yolk-plug and the rearrangement and differentiation of its constituent cells.

It is certainly noteworthy that the slit-like archenteron does not appear in the midst of the yolk-cells until the epiblast

reaches the region of the blastoporic margin ; and it is this fact, together with a predisposition to discover, if possible, an invaginative process, which seems to have led to the description of the formation of the archenteron of the *Anura* by invagination of the epiblast.

A careful examination of the walls of the extending archenteric cavity reveals no evidence in support of this ideal invaginative process ; on the contrary, it shows that even at the period of completion of the blastoporic opening (see figs. 1 and 3) the greater part of the cavity is surrounded by large nutriment-laden, marginally pigmented cells. More especially is this the case in the anterior portion of the, as yet, incomplete cavity (fig. 2), and at its posterior extremity (fig. 3), where the slit-like space is only just formed. In the latter situation the only cell that can be considered as distinctly epiblastic is that marked *c*. The remaining cells are undoubtedly large yolk-cells ; and it is only by the division of these cells, and the arrangement of some or all of their descendants into a distinct layer, that the true hypoblastic lining of the alimentary canal and its diverticula is formed. As the completion of the definite hypoblast is intimately associated with the separation of the mesoblast and chorda, its further history cannot at this period be entered upon, and we may therefore proceed to the termination of this section by a summary of the conclusions deduced from that which has been here set forth.

1. The blastopore is a deficiency in the posterior wall of the archenteron.

2. The archenteron of the *Anura* is not formed by invagination, but by a process of splitting amongst the yolk-cells very similar to that described by Houssay (26) in the *Axolotl*.

3. The situation of the archenteric cavity is first defined by the deposition of pigment in the adjacent margins of a double row of yolk-cells.

4. No portion of the archenteric wall is formed by invaginated epiblast ; on the contrary, the archenteron is surrounded



in the first phases of its development by large yolk-cells, which eventually give rise to the definite hypoblast.

5. The ventral lip of the blastopore indicates the posterior end of the primitive ventral wall of the archenteron.

6. There is no ventral and forward extension of the archenteron in front of and below the ventral lip of the blastopore by the production of a diverticulum in that situation.

7. The ventral wall of the archenteron, in front of the ventral lip of the blastopore, is completed by the extension of the anterior end of the cavity, and by the withdrawal and modification of the cells of the yolk-plug.

#### Manner in which the Anus of Rusconi closes.

Having discussed the changes that take place in the ovum up to the time of the formation of the large circular blastopore or anus of Rusconi, we will now proceed to describe the manner in which, according to our observations, the closure of the blastopore or anus of Rusconi seems to be effected, and to give in some detail an account of the structures which are the immediate result of the method of closure about to be described.

According to some of the former accounts, to which we have made reference above, the anus of Rusconi has been said to diminish in size by the gradual coming together of each portion of the blastoporic rim simultaneously. This we believe to be incorrect. No doubt from surface views alone the hitherto accepted accounts receive much support.

We have, however, paid particular attention to this point, and have come to the conclusion that the anus of Rusconi gradually diminishes in size by the concrescence of the ventral part of the lateral lips, as indicated in figs. 10 and 18. In these diagrams, the space lettered *BR*, inclosed between two concentric rings, represents the blastoporic rim at the time of the completion of the circular blastopore, or anus of Rusconi; and the shaded space, lettered *BR'*, represents in fig. 10 the position of the blastoporic rim soon after the anus of Rusconi has begun to diminish in size, and in fig. 18 a later



stage, when the anus of Rusconi has "contracted" so much that its diameter is now only about one third of its original size.

Figs. 7, 8, 9 are from sections taken nearly horizontally through an embryo in which there was no trace of neural folds, and in which the anus of Rusconi had only slightly contracted.

Fig. 10 is a diagram of the same embryo representing the changes which we conclude must have taken place.

The blastoporic rim, round which the three layers, epiblast, mesoblast, and hypoblast, are all fused, which occupied, on the completion of the circular anus of Rusconi, the position indicated by the space *BR* between the two concentric circles, had in the case of the embryo examined come to occupy the position of the shaded portion lettered *BR'* in the diagram. In the same diagram the line *x* represents that portion of the original anus of Rusconi which has become closed up by the concrescence of the ventral part of the lateral portions of the rim, as stated on p. 466.

We were unable in this specimen to find any trace of the line of fusion *x* on the surface, and as yet there was no trace of the neural folds. Accordingly, in order to be able to cut our sections as near as possible in the horizontal plane as desired, we had to dissect away the opposite pole of the embryo, and then by observing the shape of the yolk-plug we were able to cut our sections very nearly as required.

Fig. 7 has been drawn from a section (which is not quite horizontal) taken through the centre of that portion of the blastopore which was still open. At the lips of the blastopore the three layers are seen to be fused.

The line running from *ZZ'* ends at the furthest point from the blastoporic lip to which the fusion of epiblast (*EP*) and mesoblast (*ME*) extends on one side, and the line from *ZZ* ends at the furthest point from the blastoporic lip to which the fusion of those layers extends on the other side. It will be noticed that on one side the fusion extends further than on the other. No doubt this is chiefly owing to the section being not quite horizontal, the side of the *ZZ'* being the more ventral of the

two. We have, however, taken the thicker side for the purpose of measurement.

If the diminution in size of the blastopore has been produced by the concrescence of the ventral portion of the lateral lips as suggested above, and as diagrammatically shown in fig. 10, it is clear that the fusion of layers ought to extend further from the actual lip of the blastopore on the ventral border of the blastopore than on the sides or dorsal border. This will be clearly seen by reference to the diagram, fig. 10.

It is equally clear that this would not be so if the closure of the blastopore were strictly centripetal, in which case the fusion of layers should extend on all sides equally.

If the distance be measured across the blastopore in fig. 7 with a pair of compasses, it will be found to be about the same distance as between the edge of the blastopore lip and the point of furthest extension of fused epiblast and mesoblast on the side of  $ZZ'$ .

In the series of sections from which this fig. 7 and the two represented in figs. 8 and 9 were selected, the open blastopore was present in forty sections.

Assuming the open blastopore to have been circular, we may conclude that it would have required forty sections to cut through the fused mass between the edge of the blastoporic lip ( $B'$ ) and the furthest extension of the fused mass indicated by the line drawn from  $ZZ'$ , for we have just now seen that the distance from  $B$  to  $B'$  is about the same as from  $B'$  to  $ZZ'$ . In other words, the forty-first section ought to show no, or but little, fusion of layers.

Fig. 9 has been drawn from the forty-first section, and instead of there being no fusion of layers there is an extent of fused layers about equal to the two fused blastoporic rims, or, indeed, a little more.

At the sixtieth section the epiblast and mesoblast are still distinctly fused, and it is not until the eightieth section below the edge of the ventral lip of the blastopore that the layers can be definitely said to be separate. In short, whereas by calculation the fusion of layers on the lateral lips will be cut through

in forty sections, it requires close upon eighty sections to cut through the fused mass at the ventral lip.

In fig. 10 the levels of the sections (figs. 7, 8, 9) are indicated by the lines 7, 8, 9.

Thus a sagittal section through the blastopore ought to show a very much larger mass of fused layers at its ventral lip than at its dorsal.

This is seen to be the case as shown in fig. 5, which is a sagittal section through the blastopore of a rather older frog embryo. The blastopore has closed to a considerably greater extent than was the case in the embryo we have just been describing. The actual extent of the closure of the blastopore by the concrescence of the latero-ventral lips is represented, we think, by the distance between the point *x* and the present edge of the ventral lip of the blastopore.

We find by a series of measurements that at whatever stage during the closure of the blastopore the section be taken, the distance between point *x* and the edge of the dorsal lip of the blastopore is always approximately the same, and the same as the diameter of the blastopore at its first commencement. We say approximately, for, owing probably to variation in size of the egg, the measurements do not exactly agree.

Fig. 11 was drawn from a living specimen in which the blastopore had become greatly reduced, and in which the neural plate and neural groove were distinctly visible along the dorsal surface.

From the lower, or ventral, lip of the open blastopore stretches a very faint (too deeply drawn in the figure) line, which probably is the remaining trace of the line of concrescence of latero-ventral blastoporic lips.

Fig. 13 is a section taken at right angles to this line, not of the same embryo, but of one about the same age. In this the line is seen to be a groove (*PG*) on the surface, below which all these layers are fused.

We will at once call this groove primitive groove, and the fused mass below it primitive streak; for, as we hope to prove in the sequel, there can be no reasonable doubt that

these structures are the homologue of those parts in the chick embryo to which these names were originally given.

A section taken dorsal to the blastopore, and dorsal to the fusion of layers at the dorsal lip of the blastopore, is seen in fig. 12.

Here the epiblast is seen to be quite distinct from the mesoblast. There is a distinct groove, the neural groove, very different in character from that of the groove ventral to the blastopore, or primitive groove, while the epiblast is thickened on either side to form the neural plate. The level of the respective sections is marked in fig. 11 by the lines numbered 12 and 13.

Before discussing the relations between the structures, the formation of which we have been following in the last two or three pages, and which we have named primitive streak and primitive groove, and the primitive streak and primitive groove of the chick, we will follow its history a little further, until the stage at which we may say it has attained its greatest development.

We must, therefore, describe the condition of the primitive streak of a frog embryo of about  $2\frac{1}{2}$  mm. in length; that is to say, an embryo in which the neural folds are on the point of meeting, or have just met, as shown in fig. 23.

### Primitive Streak of the Frog at the Time of the Closure of the Neural Folds.

Fig. 23 is the surface view of the posterior end of a  $2\frac{1}{2}$  mm. frog embryo, in which the neural folds have just met.

It was drawn partly from a preserved specimen, and partly from a model, as described on page 453.

The line of junction of neural folds (*J*) is marked by a deep groove ending posteriorly in the blastopore.

The blastoporic opening, as seen from the surface, is no longer circular, but is more or less lozenge-shaped, due to the folding its dorsal lips have undergone (as will be described later).



The lengthening of the embryo has removed from the blastopore all trace of the "yolk-plug."

Running ventrally from the blastopore is the primitive groove, a rather narrow but now sharply defined groove near the ventral end, which is suddenly deepened into a pit.

Beyond the pit the groove continues a short distance. This pit is the commencement of the anus, which will shortly perforate at this spot. In fact, in the model from which this figure was partly drawn the anus had just perforated. In the sections, however, about to be described as representing this stage the anus has not quite perforated. On either side of the primitive groove a distinct ridge is visible.

The bracket (*PS*) in the figure includes the whole of the primitive streak from end to end.

The sections 14, 15, 16, 17, are horizontal sections taken along the lines 14, 15, 16, 17, in fig. 23, and are, therefore, taken at right angles to the longitudinal axis of the primitive streak.

Fig. 14 is a camera drawing of a section through the blastopore (line 14, fig. 23), which is nearly closed in.

In this section the epiblast, mesoblast, and hypoblast are seen to be all fused at the lips of the blastopore. It may be noted that the mesoblast, close to the lip of the blastopore, seems to be divided into two layers: a very dense compact layer (*ME'*) next the epiblast, in which the cells are so closely packed as to render it quite impossible to draw each cell by camera; while inwardly the mesoblast-cells (*ME''*) are much looser, and are more deeply pigmented and easily traceable by help of the camera. In fact, one is almost inclined to say that the former are mesoblast-cells of epiblastic origin, while the latter are mesoblastic cells of hypoblastic origin.

Fig. 15 is a section taken about midway between the blastopore and the anal pit; that is to say, across the middle of the primitive streak. This section is directly comparable to that just described, except that the lips of the blastopore have met and fused, as described in the earlier part of this paper. The surface is distinctly grooved, the edges of the

groove being raised slightly into ridges. The same feature in the mesoblast may be noticed here as in fig. 14.

Fig. 16 is taken through the anal pit.

Here the primitive groove is very much deepened, so that not more than two or three cells prevent the completion of the anal perforation. This pit is deeply pigmented, and heavy deposits of pigment line the few intervening cells between exterior and archenteron. All three layers are still fused.

Fig. 17 represents a section taken below the anal pit; that is to say, it was the thirteenth section after the last one figured, fig. 16. In this, although behind the anus, there is undoubted fusion of epiblast and mesoblast. It is altogether ventral to the archenteron, so that there can be no fusion with true hypoblast. There is no fusion either with the yolk-cells. The surface is grooved, as in the sections anterior to the anus.

The fusion of layers continues through six more sections; that is to say, there are about twenty sections ventral to the anus, in which there is fusion of epiblast and mesoblast; in other words, the anus is a perforation through the primitive streak.

### Comparison of the "Primitive Streak" of the Frog with the Primitive Streak of the Chick.

We have now described the history of the blastopore or anus of *Rusconi* from the time of its first formation until it has been reduced to a comparatively minute passage between the archenteron and exterior. We have described this change as being due to the concrescence of the lower lips of the blastopore, as shown diagrammatically in figs. 10, 18, 19, whereby there is produced a median streak characterised by the fusion of layers, along the surface of which lies a groove, stretching from the ventral lip of the remaining blastopore ventralwards as far as the original extension of the blastopore

or anus of Rusconi. To this structure and to the groove upon it we have applied the terms primitive streak and primitive groove respectively.

If one of our sections figured, e. g. fig. 15, is compared with the figure of a transverse section of the primitive streak of a chick on p. 155 of Balfour's 'Comparative Embryology,' vol. ii (second edition), the resemblance between the two sections is very marked.

In each case there is an intimate fusion between epiblast and mesoblast, and a more uncertain fusion between mesoblast and hypoblast. In each case the surface is marked by a groove.

Nor is it at all impossible that the loose pigmented cells noticed above, and lettered *ME''* in figs. 14 and 15, may be compared with Balfour's "layer of stellate cells" shown in the figure in the 'Comparative Embryology' referred to. In both cases they seem to arise from the hypoblast rather than the epiblast. In the frog, however, they are unrecognisable a short distance from the streak.

In comparing the two streaks with regard to their relations to the rest of the embryo, we find that we must use the term primitive streak in a rather wider sense than it is usually used. In the chick the anterior limit of the primitive streak may be said to be marked by the posterior end of the notochord with which the streak is fused.

The structure we have so far called primitive streak runs from the ventral lip of the blastopore. It is, however, obvious that had the concrescence continued a little further, so that the whole anus of Rusconi had been obliterated by the fusion of the blastoporic lips, the primitive streak would have then commenced at the posterior end of the notochord, as in the chick; for the notochord and neural folds in the frog are continuous with—that is to say, fused with—the dorsal lip of the blastopore. It is, therefore, clear that the homologue of the primitive streak of the chick is in the frog the whole of the blastoporic lip, whether fused or not.

### Relation of Anus to Blastopore.

The relation of the anus to the blastopore has been a much controverted subject. Goette (14) and Balfour (1) described it as an entirely separate perforation of the body-wall ventral to the blastopore; Spencer (52) stated that the blastopore became directly the anus; Sidebotham (51), who gave a most careful and exact account of the facts, agreed with Goette and Balfour on this point, and, failing to recognise the primitive streak in the frog, described it as they had done, as a new opening independent of the blastopore. In Erlanger's (10A) opinion it is a secondary opening in the situation of the posterior part of the original blastopore. Those who have followed our account so far will observe that it agrees most closely with those of Goette, Balfour, and Sidebotham on this point; but, since the perforation takes place within the primitive streak, the conclusions we draw from the facts are different. We infer, therefore, with Erlanger, that the anus of the frog, although apparently a new perforation, is really a reopening of a temporarily closed portion of the original blastopore.

Since the perforation occurs at the base of the diverticulum of the archenteron (which, it will be remembered, was formed by the closing in of the ventral portions of the lateral lips of the blastopore), it may be supposed that it is the most ventral end of the blastopore which, morphologically speaking, persists as the anus.

### The Primitive Streak of the Frog and other Vertebrates.

The term primitive streak appears to have been first applied to the dark line which appears in the avian blastoderm at the posterior part of the area pellucida. This line is generally looked upon as the optical expression of a linear thickening and fusion of the blastodermic layers, though Kölliker (31, p. 134) maintains that it is due to proliferation of the epiblast-cells alone. It seems certain, however, that it is impossible,



during certain periods of the growth of the embryo, to distinguish the three germinal layers from each other in the area of the primitive streak (Duval, 10, p. 181).

After a time differentiation proceeds in the streak; a groove appears on its superficial surface, and this is deepened anteriorly into a perforation, which ultimately becomes the neurenteric canal (47). The anterior wall of this perforation is formed by a mass of cells in which the epiblast and hypoblast are united (12). In the posterior portion of the streak the anal membrane is developed by the gradual thickening and apposition of the hypoblast and epiblast (13, pl. x, figs. 4 and 5, p. 299; and 47, pl. xiv, fig. 81, p. 203).

The lateral margins and posterior extremity of the streak are continuous with the mesoblast, which appears to grow out from them into the surrounding area.

The anterior end of the streak is continuous with the chorda ventrally, and the central part of the neural plate dorsally. In the region surrounding the primitive streak the three layers of the blastoderm are distinct from each other, except in front of the anterior extremity of the streak, where the so-called "Kopffortsatz," the first rudiment of the chorda, is still continuous with the entoderm. The connection between the hypoblast and the mesoblast, which exists throughout the whole length of the streak, is first dissolved posteriorly, where for a certain period the epiblast and mesoblast are fused, but the hypoblast forms a distinct layer.

Between the primitive streak of a bird and the frog there are resemblances and differences of importance. In the frog the primitive streak is formed by a conrescence of the lips of the blastopore, which proceeds from behind forwards, and which is only completed on the obliteration of the neurenteric canal. In the bird the primitive streak is formed from before backwards according to Duval (10) and Schwarz (47), from behind forwards according to Balfour and Deighton (2) and Koller (30); and the appearance is due apparently to thickening and fusion of the two primary layers—Duval (10), Balfour and Deighton (2).

In both the frog and the bird the lateral margins and posterior extremity of the streak are continuous with the mesoblast, which lies free between epiblast and hypoblast outside the area of the streak.

In the frog the anterior wall of the neurenteric canal is bounded by an area of fusion, in which the middle of the neural plate and the posterior end of the chorda are united. After the obliteration of the neurenteric canal the posterior end of the chorda and the centre of the neural plate are continuous with the anterior end of the primitive streak.

In the bird the anterior end of the primitive streak is at first continuous with the centre of the neural plate and the "Kopffortsatz," and after the formation of the neurenteric canal the chorda and the neural plate are fused in the anterior wall of the latter orifice, becoming again continuous with the anterior end of the primitive streak after the disappearance of the neurenteric canal.

In the frog the anus is formed in the posterior part of the primitive streak. It is a reopening of a portion of the closed blastoporic orifice. It is not the remains of the blastopore, as in *Amblystoma punctatum* (39) and *Bombinator* (15). The anus of the bird is morphologically equivalent to the anus of the frog; and it is also formed, in all probability, by a reopening of a previously closed orifice (10).

In reptiles, according to Kupffer (33), there is no primitive streak, but in the posterior part of the embryonic area the epiblast is invaginated, and the mesoblast arises, in part at least, from the margins of the invagination. The cavity of invagination is the archenteron, and the superficial opening the Urmund, which would thus entirely correspond to the anus of Rusconi in Amphibians.

But Balfour (1, p. 168), Strahl (54), Hoffmann (24), Weldon (56), Mitsuruki and Ishikawa (37), and Wenckebach (57) describe a primitive streak. Balfour states that in the primitive streak of lizards the epiblast and hypoblast are fused, though the greater part of the streak consists of proliferated epiblast. Wenckebach, however, looks upon the hypoblast as

a purely passive agent in the formation of the primitive streak in lizards; and in the tortoise, according to Mitsuruki and Ishikawa, the streak consists, in the first instance, mainly of a mass of hypoblast or yolk, which they compare to the yolk-plug of Amphibians. To this, however, it cannot correspond, for we have already shown that the yolk-plug of *Rana* is a portion of the ventral wall of the archenteron, whilst the primitive streak is formed by the fusion of the lateral lips of a deficiency in the posterior wall of the same cavity.

But, whatever the mode of its formation may be, the streak eventually becomes perforated, both anteriorly and posteriorly: anteriorly by the neurenteric canal—Balfour, Hoffmann, Weldon, and Strahl;<sup>1</sup> and posteriorly by the anus—Weldon. It is thus, in all essential respects, comparable with the primitive streak of birds.

In mammals also a primitive streak is found. It is described as commencing in the sheep (5) and in the shrew (25) as a knob-like swelling of the epiblast in the posterior part of the embryonic area. The epiblastic thickening extends backwards and terminates in a tail-swelling. According to Bonnet (5), Hubrecht (25), and Hensen (20), the two primary layers fuse in the streak, though it does not seem certain that the hypoblast takes any part in the thickening. Kölliker (31) denies that the hypoblast takes part in the formation of the streak in the rabbit. Rabl (41) agrees with Kölliker, and Fleischmann (11) makes a similar statement concerning the cat. In the primitive streak of the guinea-pig (29) there is fusion of the layers anteriorly, but posteriorly the thickened epiblastic ridge is separate from the hypoblast. In the mole (17) all the layers take part in the formation of the streak, but after a time they are fused only at its anterior and posterior ends, whilst in the middle the hypoblast forms a distinct layer. At the anterior end of the mammalian primitive streak more or less distinct traces of a neurenteric canal have been found in the rabbit by Strahl

<sup>1</sup> Strahl's statement (54), that in *L. agilis* the perforation occurs in the middle of the streak, simply means, apparently, that there is a region of fusion between the epiblast and hypoblast in front of the opening.



(55), in the mole by Lieberkuhn (35) and Heape (17), in the sheep by Bonnet (5 and 6), and in the bat by Van Beneden (3). In the anterior wall of the opening the chorda and neural epiblast are fused, and laterally and posteriorly the mesoblast hangs in connection with the margins of the streak.

The anal membrane is formed in front of the posterior end of the streak in the rabbit—Strahl (55); in the mole—Heape (17); in the guinea-pig—Keibel (29); and in the rat and mouse—Robinson (43). In the sheep, Bonnet (7) states that the anal membrane is situated at the posterior end of the streak; and Rabl (41) figures it in the same position in the rabbit.

The differences described are slight and probably unimportant, and the main facts stand out clearly. As in the Sauropsida, the primitive streak is a line of fusion amidst the germinal layers. It is continuous anteriorly with the neural epiblast and the chorda, laterally and posteriorly with the mesoblast of the surrounding areas. It becomes perforated by an evanescent neurenteric canal and by a permanent anal orifice.

In the Cyclostomata the posterior part of the blastopore remains open as the anus. In the ventral lip of this orifice the epiblast alone is at first differentiated, the hypoblast and the mesoblast remaining fused until a comparatively late period—Goette (15) and Kupffer (34). The blastopore is closed from before backwards according to Goette's (15) statement, and consequently there is formed in front of the anus a mass of cells called the "Teloblast" by Kupffer (34). This mass of cells remains for a time fused with both epiblast and hypoblast (Goette, pl. iv, fig. 41), but afterwards it separates from the superficial epiblast and the hypoblast (Kupffer, pl. xxviii, fig. 28), but remains continuous in front with the chorda and neural tube. Evidently the area from the front of the anus to the posterior end of the chorda and neural tube in *Petromyzon* corresponds closely to the anterior portion of the primitive streak of the Sauropsida and mammals. It is never perforated by a neurenteric canal, but the absence of this passage has no important bearing, as it has probably been suppressed.



In Teleostean fishes an area of fusion is found round the lips of the blastopore (Henneguy, 18), but the fusion in front of the blastopore is much more extensive than that on the sides and posteriorly, and in its anterior part a vesicle appears—"Kupffer's vesicle." In front of Kupffer's vesicle the chorda and neural plate are fused, and the margins of the fused area are continuous with the mesoblast. Eventually the posterior portion of the blastopore is closed by the fusion of its lips (18, p. 502). The position of the anus is not definitely stated. The fused area corresponds in the Teleostei even more closely than in the Cyclostomata with the anterior portion of the primitive streak of the Sauropsida and mammals, for it is partially perforated anteriorly by Kupffer's vesicle, which is evidently situated in the position of the neurenteric canal of the higher Vertebrata.

We have before referred to Balfour's description of the condition of the posterior end of the embryo in the Elasmobranchii (p. 11), and to this we have only to add that at a later period the ventral part of the blastopore also becomes closed by fusion, and that at a subsequently later period the anus is formed as a secondary opening in the line of fusion (Schwarz, 47).

The observations upon the Ganoids are not sufficiently complete to afford any definite basis for comparison, but it appears (see Balfour, vol. ii, pp. 84—86, and the extract in Hoffmann and Schwalbe's 'Jahresbericht' for 1878, p. 222) that after the segmentation and during the formation of the archenteron the blastopore becomes distinct, first at its dorsal lip and then in its whole circumference; it ultimately closes, and Salensky (44) states that the anus is produced afterwards in the situation which was first occupied by the blastopore. There is, however, no evidence as to whether the blastopore in Ganoids closes from before backwards, as in Teleostei and Elasmobranchii and Cyclostomes, or from behind forward as in the *Rana temporaria*.

The primitive streak of the Amphibia appears during the period of extension of the archenteron. It is formed by a fusion

of the layers in the lips of the blastopore and by conerescence of the lips of the blastopore, either from before backwards as in Triton (Goette, 15), or by fusion of the lateral lips of the blastopore between the neurenteric canal and the anus, arriving at its completion on the obliteration of the neurenteric canal, as in Bombinator (15) and *Amblystoma punctatum* (39); or it may be formed, as we have already shown in *Rana temporaria*, by fusion of the lips of the blastopore from behind forwards.

In Teleosteans and Cyclostomes it is formed by fusion of the blastoporic lips from before backwards, also whilst the archenteron is being formed and extended, and in Elasmobranchs by fusion of the lateral lip of the blastopore behind the neurenteric canal.

In the Sauropsida and Mammalia we have no definite proof of a primary blastoporic opening, for the aperture so named by van Beneden in the rabbit (4), and by Selenka in the opossum (50), is not yet definitely located; but the primitive streak in these Vertebrates is readily comparable with the secondary condition of the Amphibian blastopore as formed in *Rana temporaria*. It is an area of fusion of the germinal layers which afterwards becomes perforated by the formation of the anus. The appearance of the fused area at a comparatively late stage in reptiles, birds, and mammals—that is, after the archenteron is well established (if we except Kupffer's views on the function of the archenteron of reptiles, according to which the primitive gut is produced by invagination)—throws but little difficulty in the way of the comparison, for it is most probably a mere heterochronous displacement associated with the precocious segregation of the hypoblast in the higher Vertebrates.

Therefore, if we use the term primitive streak in the sense in which it is used in the description of the avian blastoderm—that is, as a term which signifies an area of fusion of the blastodermic layers which is continuous laterally and posteriorly with the separated epiblast, mesoblast, and hypoblast, and is in front continuous with the neural plate and chorda, and

which is perforated posteriorly by the anus, and may be perforated, more or less completely, anteriorly by the neurenteric canal,—then we are bound to admit that this region and the corresponding areas in mammalian and reptilian blastoderms are the homologues of the primitive streak of *Rana temporaria*; and conversely we are forced to conclude that, as the primitive streak of *Rana temporaria* is formed by the linear fusion of the undifferentiated area in the lips of the blastopore, the primitive streak of the Sauropsida and Mammalia is homologous with the fused lip of the blastopore of *Rana*.

Strictly speaking, therefore, the typical primitive streak is an area which extends antero-posteriorly from the point at which the fusion of layers commences, in front of the anterior lip of the blastopore (fig. 10, *Z*), to the point at which the fusion of layers terminates behind the posterior lip of the blastopore (fig. 10, *Z'*); and laterally from the termination of the fusion on the left lip of the blastopore (fig. 10, *ZZ'*), to the termination of the fusion of the layers in the right lip of the blastopore (fig. 10, *ZZ*).

If this is the case, then it is evident that the primitive streak of Bombinator, Triton, and Petromyzon, as usually described, is not homologous with the primitive streak of *Rana temporaria*, the Sauropsida and Mammals, but only with that portion of it which lies in front of the anus. And it is also evident that the primitive streak of Teleosteans and Elasmobranchs, after the complete closure of the blastopore, is the exact homologue of the typical primitive streak as formed in birds.

With regard to the Ganoids, it is only possible to say that they seem to closely resemble the Teleosteans. It will be noticed that we have made no reference to Amphioxus. With regard to this somewhat anomalous Vertebrate, we can only observe that so far as the observations of Hatschek (16) and Kowalevsky (32) go, there is no primitive streak formed, unless we accept in full the concrescence theory, and look upon the dorsal axial line as its representative; but even if we adopt this theoretical conception, of which there is no



positive proof, we are still impressed by the fact that the anus is neither the remains of the blastopore, nor is it formed secondarily by reopening of the fused lips of the blastopore, but it appears as a new formation below and in front of the blastoporic opening; at least Hatschek figures it in that situation, and his description is as follows:—"Die Unterbrechung der Communication zwischen Darm, und Medullarrohr erfolgt ungefähr gleichzeitig oder auch etwas später als der Durchbruch des Afters. Der After bricht ventralwärts von dieser Communications-öffnung, die den letzten Rest des Gastrulamundes repräsentirt" (16, p. 79).

The anus of the frog and many other Vertebrata bears a similar relation to the neurenteric canal, but in no other Vertebrate except Amphioxus is the anus a distinctly new formation; on the contrary, it is very evidently an aperture formed by the reopening of a temporarily closed orifice, or by perforation of the homologue of that orifice. In view of this fact, it seems very probable that future observations will modify Hatschek's results, and remove the contradiction which at present exists between Amphioxus and other Vertebrata. If, however, this proves not to be the case, it will then have to be decided whether the condition which is found in Amphioxus, or that which is so general amongst the other Vertebrata, is the more primitive.

#### The Fate of the Primitive Streak in the Frog.

In discussing the primitive streak of the frog it will be convenient to consider separately—

(I) The fate of the ventral moiety.

(II) The fate of the dorsal moiety.

Goette (15) has pointed out that in *Petromyzon* the homologue of the primitive streak must be the whole rim of the blastopore. Apparently, however, he does not extend the same reasoning to the case of the frog. For in that case, according to his description, and to the figure he gives, in which he indicates the position of the primitive streak by a bracket, he does not include the ventral lip of the anus.



To quote his words, "Das Homologon des Primitifstreifs ist das die ventrale Hälfte des Schwanzes von seiner Spitze bis zum After" (Haut, ventrale Hälfte des Schwanzdarms und der Mesodermplatten, Hinterwand des Aftersdarms).

It is a question how far we ought to speak of the derivatives of the primitive streak being "das Homologon" of the primitive streak. Is it quite correct to say that the adult frog is the homologue of the egg from which it has been developed?

However, it is clear that Goette means that the ventral half of the tail is derived from the primitive streak. Here we must again differ from him.

As the result of our observations we conclude that not only the ventral half of the tail is derived from the primitive streak, but also the dorsal half as well, with the exception of nearly the whole of the skin of the tail.

This we shall hope to show while considering the fate of the dorsal moiety of the primitive streak.

We now proceed to describe the fate of the ventral moiety subsequent to the stage corresponding to figs. 23, 15, 16, 17.

Figs. 20, 21, 22, are camera drawings of sections of the posterior end of a  $4\frac{1}{2}$  mm. tadpole, taken at levels corresponding to those of sections 15, 16, and 17 respectively (vide lines 20, 21, 22 in fig. 26, and lines 15, 16, 17 in fig. 23).

In each case the drawings have been made by camera, and from unstained sections, and the natural appearance as regards tint and shape has been reproduced as nearly as we were able to reproduce it.

In figs. 20, 21, and 22, there is no trace of a primitive streak, but all three germinal layers are now distinct and separate. In fig. 22 the mesoblast *ME* may be said to be still fused with the epiblast *EP'''*, but it is, nevertheless, quite distinct in character of cell, and in degree of pigmentation. In the two other sections, figs. 21 and 20, the mesoblast has become entirely separated from the epiblast.

By comparing these three sections with the sections from

which figs. 15, 16, 17 were drawn, the actual fate of the primitive streak in this region may be fairly easily determined.

In comparing fig. 20 with fig. 15, the change that has taken place in the former seems to be of this nature. The three layers, epiblast, mesoblast, hypoblast, instead of being "fused," are now (fig. 20) easily distinguishable and completely apart.

This fusion of layers we must most probably interpret as indicating an area of proliferation of cells, which indeed is a characteristic feature of a primitive streak; and when this proliferation of cells ceases, the primitive streak may be said to be no longer of physiological importance, though the area of its former extension, being of morphological interest, should be noted.

Thus, if we are regarding the primitive streak from a physiological point of view, we must say that this portion has now ceased to exist. If we regard it from a morphological point of view, we may say that this portion has ceased to be "functional," but, nevertheless, includes the area in the bracket labelled *PS* in fig. 26 as being within the limits of the original extension of the primitive streak. This portion we have attempted to render more distinct in fig. 26 by a different method of shading and crossed lines, *PS' PS'''*.

The fate of the ventral moiety may be understood by reference to fig. 26, combined with a comparison of figs. 20, 21, 22 with figs. 15, 16, 17. By words we may explain its fate by saying that the ventral moiety of the primitive streak splits up into portions of the three germinal layers.

(I) Laterally and ventrally into (*a*) the posterior extremity of the mesodermal plate.

(II) In the median plane into two layers, (*b*) an outer or epiblastic layer, forming part of the skin, and (*c*) an inner or hypoblastic layer of large darkly pigmented cells, fig. 20, *HY'*, forming the hind wall of the rectal spout.

The difference in character of cell between the posterior wall and anterior wall of the rectal spout is very marked, as

shown especially well in fig. 20. This feature is not so marked in stained specimens, where the degree of pigmentation does not show up so well.

This description so far agrees with Goette (15), and Schwarz (47), who follows Goette in this respect, except that we include the ventral lip of the anus within the primitive streak, which apparently these authors do not, though for what reasons it is not easy to understand.

### Fate of the Dorsal Moiety of the Primitive Streak, and Development of the Tail.

In the dorsal moiety the fate of the primitive streak is different.

Instead of splitting up, it remains as a proliferating area; or according to our definition above, this portion of the primitive streak remains for a longer time "functional."

The relation of the neural folds to the blastopore, that is to say to the primitive streak, must be necessarily considered in connection with the fate of the dorsal moiety of the primitive streak.

Reference to fig. 14, which passes through the rapidly closing blastopore (*BL'*), conclusively proves that there is here no trace of neural folds. The epiblast shows no signs whatever of any thickening, the mass of cells being merely the fused layers—that is to say, the undifferentiated cells at the lips of the blastopore—of typical primitive streak.

There is no trace of neural folds—that is, of thickened epiblast separate from mesoblast—until several sections anterior to the still open portion of the blastopore. That is to say, that which from a surface examination appears to be the lower end of the neural folds, is really the dorsal portion of the lateral lips of the blastopore folded up along with the true neural folds.

If this were not so, there would be a sudden break at the posterior end of the neural folds; and the neural canal, if it opened anywhere, would open to the exterior dorsal to the blas-

topore, and not into the blastoporic canal, as is well known to be the actual case.

This we have tried to make clear by the diagrams, fig. 18 and fig. 19.

In these diagrams the external surface of the neural plate has been dotted *NP*. The space *BR* between the two small concentric circles represents the position of the fused layers—that is to say, the lips of the blastopore at the time of the first formation of the blastopore.

The shaded space *BR'* represents the position of the same fused layers in fig. 18 at the time of the complete formation of the neural plate, but before it has commenced to fold up; in fig. 19 after the neural plate has become completely folded, and its outer lateral edges are just meeting.

*BL'* is that portion of the blastopore which remains open longest.

The two asterisks in fig. 18 mark the two lines of epiblast immediately adjoining the lateral edges of the neural plate.

When the neural plate has become folded, and when its lateral edges have met and fused, and separated from the adjoining epiblast, the lines of epiblast indicated by the asterisk will have fused and made good the gap which would otherwise be caused in the skin by the separation from it of the neural plate.

In fig. 19 the folds are represented as having nearly met, so that the outer surface (dotted) of the neural plate is now no longer seen, except a narrow strip through the now rapidly approaching lateral edges of the neural plate.

The daggers similarly mark two spots in the epiblast adjoining the fused layers at the outer edges of the lateral lips of the still open portion of the blastopore.

In fig. 18 the relation of the neural plate to the dorsal lip of the blastopore—that is to say, to the dorsal end of the primitive streak—is clearly represented.

In fig. 19 the folding up of the neural plate is nearly complete, and with it the dorsal part of the primitive streak—that is to say, the dorsal portions of the lateral lips of the



blastopore have been folded up along with the neural folds, and the adjoining areas of epiblast marked by the daggers will shortly meet and fuse, just as will the areas of epiblast adjoining the lateral edges of the neural plate marked by the asterisks.

Similarly, just as after the meeting and fusing of the epiblast along the lateral edges of the neural plate has taken place, and just as the neural plate—or tube, as it now will be—separates from and lies within the skin, so also will that portion of the primitive streak separate from the skin and come to lie within the embryo.

The posterior or ventral portion of the primitive streak does not become folded in this way, for by the time the folding of the dorsal portion has been completed and the separation from the skin has taken place, the lower or ventral portion has, as we have described above, split up and ceased to exist as a “functional” primitive streak.

By this means the primitive streak loses all direct connection with the surface epiblast, though the connection of the primitive streak with the surface epiblast may be said to be in reality retained by the direct continuity of the cells of the primitive streak with the interior lining (i. e. epidermic epiblast) of the neural tube.

The semi-diagrammatic sagittal sections, figs. 24, 25, and 26, will aid in rendering clearer the above statements.

Fig. 24 is an early stage, only a little older than the stage represented by fig. 18.

The “primitive streak,” or area of fused layer which is cut through in these three sections, is shown by a diagonal shading.

In fig. 24 the mass of primitive streak is seen to be much greater ventrally than dorsally; the extent of closure of the original blastopore is expressed by the distance between *x* and the inner edge of the lip bounding ventrally the still open portion of the blastopore.

In fig. 25 the neural plate has become folded upon itself and fused, but the neural tube so formed has not yet separated

from the skin,—it is, in fact, on the point of so doing; it therefore represents a stage rather later than that of fig. 19.

Ventrally, the primitive streak is in the middle line divided into a post-anal ( $PS'$ ) and pre-anal portion ( $PS''$ ) by the deepening of the primitive groove at one spot ( $A$ ), which shortly after completely perforates and forms the permanent anus. As we have stated above, we regard this as practically a reopening of the ventral part of the original blastopore. This is also shown diagrammatically in fig. 19,  $A$ .

Dorsally, we get a portion of primitive streak ( $PS''$ ) continuous with the dorsal wall of the archenteron ( $HY$ ), the notochord ( $CH$ ), and floor of the neural tube, which is the actual dorsal lip of the blastopore, and is exactly the same as that portion of primitive streak which bears the same relations to the same structures—dorsal wall of archenteron ( $HY$ ), notochord ( $CH$ ), floor of neural tube ( $NP$ )—in fig. 24.

Externally to this and posteriorly the section cuts another portion of primitive streak ( $PS^{iv}$ ), which is the dorsal part of the lateral lips of blastopore which have been folded up along with the neural folds, as we have already described, and as we have endeavoured to represent diagrammatically in fig. 19.

By this means a canal is formed leading from the archenteron to the neural tube, known as the neurenteric canal, which is therefore the anterior portion of the blastopore ( $BC$ ), together with the canal ( $PSC$ ) bounded ventrally by the anterior portion of the primitive streak ( $PS''$ ), and laterally and dorsally by the folded-over anterior portions of the lateral lips of the blastopore ( $PS^{iv}$ ).

At this period there is still a direct passage to the exterior from the archenteron, as well as into the neural tube; but as the folding up of the dorsal portion of the primitive streak progresses, combined probably with the continued concrescence of the more ventral portion of the lateral lips of the blastopore, this external opening—that is to say, that portion of the original blastopore which apparently remains open for the longest period—is entirely and finally closed from the exterior.

By the time this has occurred, or shortly after, the neural

tube has become separated from the skin, and with it the dorsal portion of the primitive streak.

The primitive streak should, however, still be connected with the skin ventrally by that portion which does not become folded up and nipped off as does the dorsal portion, together with the neural tube.

Possibly this connection may exist for a very short time, but practically the separation from the skin of the dorsal moiety takes place contemporaneously with the splitting up of the ventral moiety, so that the space between the dorsal moiety of the primitive streak and the skin from which it has separated, and the space between the posterior wall of the rectal spout and post-anal gut on the one hand, and the skin at the same level caused by the splitting up of the primitive streak of that area, become confluent at the point in fig. 26 just where the line *PS''* crosses the space between primitive streak and skin.

Thus it comes about that the dorsal portion of the primitive streak, which remains "functional" and gives rise to the greater part of the tail, comes to lie entirely within the embryo.

Fig. 26 represents the stage at which the tail has definitely begun to grow. The dorsal moiety of the primitive streak is seen to be lying within the embryo, and it is by proliferation of its cells that the whole of the tail is formed with the exception of the skin. In no section after (the stage shortly antecedent to this) have we been able to see a fusion between the skin and the primitive streak, though the skin lies closely over the primitive streak.

The skin would seem to grow, not at any one point, but over its whole surface, on account of the pressure caused from within by the growth of the main axis of the body, in response to which the skin must either grow or rupture.

To a certain extent the skin of the middle line of the ventral surface of the tail is derived from the primitive streak; that is, from the ventral moiety by the splitting up of the latter. This portion is distinguished in fig. 26 by a different mode of shading; but with the exception of this, which we think extends only a



short way, no part of the skin of the tail is derived from the primitive streak.

The neurenteric canal is undoubtedly to be regarded as the most dorsal part of the blastopore; and, although it remains open for a short time after the commencement of the tail, it gradually closes, as we have shown in the semi-diagrammatic figure, fig. 26, *NU*. This closing may, perhaps, be not incorrectly spoken of as the continuation and completion of the conecrescence from behind forwards which caused the closure of the ventral portion of the original blastopore.

As far as we have been able to observe, the post-anal gut only exists during the persistence of the neurenteric canal.

In fig. 26 the part of primitive streak *PS*<sup>iv</sup> has been drawn too large proportionately to the part *PS*<sup>''</sup>.

#### Conclusions as regards the Primitive Streak; its Origin and Fate.

A structure exactly comparable to the primitive streak in the chick, median and grooved, is formed in the frog (*Rana temporaria*) by conecrescence of the lips of the blastopore from behind forwards.

The anus perforates the posterior or ventral end of the primitive streak, being a deepening of the primitive groove. It may, therefore, be regarded as the reopening of the most ventral part of the blastopore.

The portion of the primitive streak with which the dorsal wall of the archenteron, notochord, and floor of the neural tube are continuous is the most anterior limit of the primitive streak, and is the dorsal or most anterior lip of the blastopore.

The neurenteric canal, which is bounded anteriorly by the dorsal lip of the blastopore, is therefore the most anterior portion of the blastopore.

The ventral moiety of the primitive streak shortly after the perforation of the anus ceases to exist, or, as we have preferred to term it, ceases to be "functional," and splits up.

The dorsal moiety of the primitive streak becomes folded upon itself like, and along with, the neural plate, and becomes



separated from the skin, and, remaining "functional," gives rise to the whole of the tail with the exception of the greater part of the skin.

We cannot find at any time any trace of blastopore or primitive streak anterior to any part of the neural plate or tube.

### The Formation and Separation of the Mesoblast.

After the transformation of the superficial layer of the yolk-cells into epiblast is completed the remainder of the yolk may be looked upon as hypoblast and modified hypoblast, for it eventually becomes transformed, partly into the true hypoblastic lining of the enteric cavity, and partly into mesoblast and chorda. It is separated from the epiblast, except at the margins of the blastopore, and it never again, in *Rana temporaria*, becomes fused with the epiblast in front of the blastopore, after the manner described by Schultze (45) in *Rana fusca*.

During the completion of the archenteron, and before the blastopore is closed, the mesoblast, in front of the blastopore, begins to separate from the true hypoblast along the dorso-lateral aspects of the archenteron by a process of delamination.

A slit-like space, which rapidly distends, appears between the hypoblast and mesoblast in these regions (fig. 12, *H*), whence it gradually extends both towards the dorsal and towards the ventral middle lines; but considerably before these clefts reach near the mid-dorsal line two sagittal clefts appear. The latter clefts separate the chorda in the middle line from the mesoblastic plates laterally (fig. 12, *S*, and fig. 6, *S*).

At this period the chorda is continuous with the hypoblast, and the mesoblastic plates and the hypoblast are still fused just outside the chorda, as well as on the ventral aspect.

Along the lines of attachment of the mesoblastic plates to the hypoblast, at the sides of the chorda, slight depressions are noticeable (fig. 6, *D*), which appear to indicate a continuation

of the archenteric cavity into the mesoblast in the manner suggested by O. Hertwig (21).

Eventually the mesoblast in front of the blastopore is entirely separated from the hypoblast, first dorsally and then ventrally, the ventral fusion remaining in the region of the liver for a considerable time.

These changes do not take place simultaneously, but appear to proceed from before backwards, so that the mesoblast is still adherent to the chorda and the hypoblast, in the posterior portion of the embryo, for a short time after the separation has been completed more anteriorly.

### The Primitive Streak Mesoblast.

The fusion of all the germinal layers in the lips of the blastopore continues after the margins of the orifice have concresced, and there is, therefore, behind the neurenteric canal an axial rod of tissue, which is grooved upon its upper surface, and which is not distinguishable into definite layers (fig. 15).

We have not been able to find in *Rana temporaria*, before the formation of the anus, any separation of the constituent parts of this axial rod into layers in the manner described and figured by Erlanger (10A). On the contrary, we find that the anus is formed as a perforation through the fused mass. But, after the separation of mesoblast from hypoblast has extended as far as the margins of the primitive streak, we find that the lower cells of the mesoblast are more loosely arranged, and that they are of more rounded form than the more superficial cells. When these lower more rounded cells are followed towards the streak they are seen to be continuous with the hypoblast of the primitive streak. The upper and denser layer of the mesoblast, traced in the same direction, is found to be continuous with the epiblast (fig. 15).

These appearances suggest the idea that the mesoblast of this region is formed partly from the epiblast and partly from the hypoblast, but there is no definite proof that such a double formation actually occurs.

## The Chorda Dorsalis.

We have already stated that in *Rana temporaria* the chorda is formed from the yolk-cells which lie beneath the neural groove. It is unimportant whether we consider these cells to be mesoblastic or hypoblastic, but we wish to emphasise the fact that the chorda in *Rana temporaria* is not formed from a layer of mesoblast which has previously separated from the hypoblast. We have been unable to find in *Rana temporaria* any appearances which would justify the conclusions which Schultze forms from his observations upon *Rana fusca*, i. e. "Dass schon auf dem Stadium der beginnenden Gastrulation in der dorsalen Urdarmwand drei Keimblätter existiren" (45).

At a later stage, however, just about the time when the medullary groove is only appearing in the posterior part of the embryo, we have noted, under the low power, an apparent separation of the mesoblast as a distinct layer across the mid-dorsal line; but this appearance we have only seen in ova which have been treated with staining reagents, and on examination with higher powers we have never been able to convince ourselves that the line of separation was normal, for we have invariably found in the space between the mesoblast and hypoblast broken fragments of cells, and we are therefore inclined to the belief that the separation of the layers was in these cases artificial, and that it had been produced by the reagents used in staining.

In unstained specimens the appearances seen in the mid-dorsal line at the time of the appearance of the medullary furrow are represented in fig. 12. The epiblast and mesoblast are separated by a distinct space (*E*). Laterally the hypoblast and mesoblast are also distinct from each other (*H*), but along the dorsal wall of the archenteron the mesoblast is neither separated from the chorda, though there are traces of the commencement of the separation on the right side (*S*), nor from the hypoblast, and the chorda is fused both with mesoblast and hypoblast.



At a later period the mesoblastic plates become entirely separated from the dorsal hypoblast, and afterwards the chorda also is separated; it then forms a distinct rod, which lies free below the floor of the neural groove and above the dorsal hypoblast except at its posterior extremity, where it terminates in the mass of cells which form the anterior wall of the neurenteric canal.

Before the commencement of the separation of the chorda from the hypoblast there are, here and there, appearances which indicate a projection of the archenteric cavity into the mass of chorda-cells, in some cases merely as a cleft-like space, in others as a more distinct diverticulum.

In *Rana fusca* O. Schultze found a peculiar secondary fusion of the mesoblast with the epiblast in front of the dorsal lip of the blastopore, "bildet sich gegen ende der Gastrulation von der Mitte der dorsalen Urmundlippe aus eine nach dem Kopfe hin vorwärts schreitende lineare Verwachsung des äusseren und mittleren Blattes aus" (45). This line of fusion he compares to the primitive streak.

We have already said that in *Rana temporaria* there is no true layer of mesoblast along the dorsal axial line in front of the blastopore, and that in this situation the chorda is formed by differentiation of the yolk-cells; it follows, therefore, that if the fusion occurs in *Rana*, it will be between the last-mentioned cells and the epiblast.

We have examined our sections carefully with reference to this point, and we find that the epiblast after its separation from the yolk-cells does not again fuse with the subjacent layer in the dorsal axial line in front of the blastopore.

It will be remembered that the separation of the epiblast terminates at a distance of  $\cdot 352$  mm. (p. 462) in front of the dorsal lip of the blastopore, that is, at the commencement of the primitive streak. In other words, the dorsal lip of the blastopore has an antero-posterior length of  $\cdot 352$  mm., and during the early stages this length does not increase; therefore there is no forward extension of the primitive streak in front of the blastopore.



The mere fact that in *Rana temporaria* the distance from the dorsal margin of the blastopore to the point at which the epiblast is separated as a distinct layer remains constant throughout the earlier stages is not a proof that the blastoporic lips undergo no concrescence from before backwards, but it is a proof that the epiblast after its separation does not again fuse with the subjacent layer.

### The Formation of the Cœlom.

Along the line of attachment of the mesoblastic plates to the hypoblast, at the sides of the chorda, it is possible to find at irregular intervals small evaginations from the archenteron (fig. 6, *D*), but these small diverticula cannot be traced for any great distance into the mesoblastic plates.

They remain for a time as blind diverticula, and then they disappear. So far as we have been able to discover they do not communicate with the cœlom, and the cœlom is not formed by extension of the archenteric diverticula, as in *Amphioxus*, but by a splitting of the mesoblast which first occurs laterally, and then extends dorsally and ventrally as in the higher Vertebrata.

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## EXPLANATION OF PLATES XXXIV & XXXV,

Illustrating Dr. Arthur Robinson’s and Mr. Richard Assheton’s paper on “The Formation and Fate of the Primitive Streak, with Observations on the Archenteron and Germinal Layers of *Rana temporaria*.”

### *Alphabetical List of Reference Letters for all the Figures.*

*A.* Anus. *a.* Anterior extremity of archenteron. *AP.* Post-anal gut. *AR.* Archenteron. *AR’.* That portion of the archenteron the posterior wall of which is formed by the closure of the blastopore. *B, B’.* Rim of blastopore lip. *b.* Dorsal lip of blastopore. *BC.* Blastoporic canal. *BL.* Blastopore. *BL’.* Portion of original blastopore not yet closed. *BR.* Original position of fused layers, that is to say, lips of blastopore at the time of the first formation of the circular blastopore. *BR’.* Position of fused layers or lips of blastopore after concrescence to a greater or less extent of the lateral lips of the original blastopore. *BR’’..* The dorsal portion of the lateral lips of the blastopore folded over with the neural plate, so that that which was their ventral or inner surface

is now seen from without. *c*. Ventral lip of blastopore. *CH*. Notochord. *D*. Diverticulum from archenteron towards mesoblast. *d*. Line of section of Fig. 2. *E*. Space between epiblast and mesoblast. *EP*. Epiblast. *EP'*. Epidermic layer of epiblast. *EP''*. Nervous layer of epiblast. *H*. Slit between hypoblast and mesoblast. *HY*. Hypoblast. *HY'*. Primitive streak of hypoblast. *J*. Line of fusion of the folded-up edges of the neural plate. *ME*. Mesoblast. *ME'*. Epiblastic or outer layer of mesoblast of primitive streak. *ME''*. Hypoblastic or inner layer of mesoblast of primitive streak. *NC*. Cranial nerve. *NG*. Neural groove. *NP*. Neural plate. *NS*. Roof of spinal cord. *NT*. Central canal of spinal cord. *NU*. Neurenteric canal. *P G*. Primitive groove. *P S*. Primitive streak. *P S'*. The part of the primitive streak ventral to the anus, i. e. ventral lip of original blastopore. *P S''*. The part of the primitive streak which is continuous with the posterior end of the notochord, i. e. dorsal lip of original blastopore. *P S'''*. The part of the primitive streak which lies between that portion of the blastopore which remains open longest and the anus, i. e. the greater part of the fused lateral lips of the original blastopore. *P S<sup>iv</sup>*. The part of the primitive streak which is folded up with the neural folds and forms the posterior wall of the neurenteric canal. *P S<sup>c</sup>*. The dorsal portion of the neurenteric canal. *S*. Line of separation of mesoblast from chorda. *S G*. Segmentation cavity. *x*. The lowest extremity of that part of the archenteron which is bounded posteriorly by the fused lips of the blastopore. *Y*. Yolk-cells. *Y P*. Yolk-plug. *Z, Z', ZZ, ZZ'*. Furthest points from the edge of the blastopore, at which a distinct fusion of germinal layers is perceptible. *\*\**. Two points of epiblast beyond the neural plate, which meet and fuse when the folding up of the neural plate is completed. *††*. Two points of the epiblast beyond the dorsal portion of the primitive streak, which meet and fuse when that portion of the primitive streak is folded up with the neural plate.

FIG. 1.—Sagittal section of a portion of the ovum represented in Fig. 4, showing the dorsal lip of the blastopore and the area of fusion in front of it. The cavity of the archenteron, and the pigmented streak in front of it which indicates the line of extension.  $\times 50$ .

FIG. 2.—A transverse section through the pigmented area in front of the archenteric cavity. The section is taken in the direction of the line *d* in Fig. 1.  $\times 115$ .

FIG. 3.—A sagittal section through the posterior lip of the blastopore of the ovum represented in Fig. 4.  $\times 115$ .

FIG. 4.—A sagittal section of an ovum at the period of completion of the blastopore.  $\times 23$ .

FIG. 5.—A sagittal section of an ovum after the disappearance of the segmentation cavity, and when the blastopore is partially closed.  $\times 23$ .

FIG. 6.—A portion of a transverse section of an ovum after the separation

of the chorda from the mesoblast, but before the complete separation of the mesoblast and chorda from the hypoblast.  $\times 138$ .

FIG. 7.—A nearly horizontal section through the posterior part of an embryo in which the blastopore had only slightly diminished. The section is taken through the centre of that part of the original blastopore which still remains open. Drawn with camera,  $\times 30$ .

FIG. 8.—A nearly horizontal section through the same embryo, but taken ventrally to the existing blastopore through the ventral portion of the original blastopore, the lateral lips of which have coalesced.  $\times 30$ .

FIG. 9.—A nearly horizontal section through the same embryo, but taken ventral to both Figs. 7 and 8. It was the forty-first section ventral to that of Fig. 7.  $\times 30$ .

FIG. 10.—A diagram to illustrate the changes that had taken place in the shape of the blastopore, in the embryo of which Figs. 7, 8, and 9 are sections. The lines numbered 7, 8, 9 are drawn about the levels at which the sections bearing those numbers are taken. The space lettered  $BR$ , enclosed by the two small concentric circles, represents the position and extent of the fused layers of the blastoporic rim at the time of the first formation of the circular blastopore or anus of Rusconi; the shaded space  $BR'$  represents the position and extent of the fused layers at the stage of the embryo of which Figs. 7, 8, and 9 represent sections. The line lettered  $x$  represents the extent of the closure of the ventral portion of the lateral lips up to this stage. The letters  $Z, Z', ZZ, ZZ'$ , refer to the same spots as those letters do in Figs. 5 and 7.

FIG. 11.—A surface view of the posterior end of an embryo, in which the blastopore has diminished considerably, but in which it is still circular. The neural plate,  $NP$ , is distinctly visible. The line  $PG$  is the primitive groove, at this period only with difficulty made out in surface view. In the figure it is drawn too distinctly. The levels at which the sections Figs. 12 and 13 were taken are indicated by the lines numbered 12 and 13. These sections (Figs. 12 and 13) were not taken from the same embryo from which Fig. 11 was drawn. This drawing was made from a living embryo.

FIG. 12.—A transverse section through the posterior portion of the neural-plate region, showing the commencing separation of the mesoblast from the chorda.  $\times 115$ .

FIG. 13.—A transverse section through the posterior part of the primitive streak of an embryo, about the same age as that represented in Fig. 5. The section is taken along the line  $x$  in the latter figure.  $\times 50$ .

Figs. 14, 15, 16, and 17 are horizontal sections through the posterior end of the same embryo, the stage being precisely the same as that of Fig. 23, on which the levels of the four sections are shown by the lines 14, 15, 16, and 17. Fig. 14 is taken through the centre of the nearly closed "blasto-



pore," and the sections 15, 16, and 17 are the 16th, 32nd, and 44th sections respectively below section 14.

Fig. 14. A horizontal section through the posterior end of the embryo, and therefore a section transverse to the longitudinal axis of the primitive streak. The section is taken through the centre of the small part of the original blastopore which is still open, which, however, is seen to be on the point of closing. The three germinal layers are fused at the blastoporic lip, and two layers of the mesoblast may be distinguished—a denser outer or epiblastic layer,  $ME'$ , and a looser inner or hypoblastic layer,  $ME''$ . The cells of the latter are further characterised by being rounder and more deeply pigmented. Drawn with camera,  $\times 55$ .

Fig. 15.  $2\frac{1}{2}$  mm. Tadpole. A horizontal section parallel to the preceding section, taken through about the centre of the primitive streak. It presents practically the same features as the preceding figure, except that at this point the blastopore has completely closed, and the opposite lips have fused, forming a typical primitive streak. Camera drawing,  $\times 55$ .

Fig. 16.  $2\frac{1}{2}$  mm. Tadpole. Horizontal section through the posterior part of the embryo. This section passes through that portion of the primitive streak in which the anus will shortly appear. The primitive groove is at this point very much deeper, forming that which is usually called the proctodæum; but complete perforation has not yet occurred. The line of perforation is, however, foreshadowed as it were by a deep irregular line of pigment. Camera drawing,  $\times 55$ .

Fig. 17.  $2\frac{1}{2}$  mm. Tadpole. A horizontal section of posterior end of an embryo ventral to the anus. This is the thirteenth section behind the anus. The primitive groove is still well marked. As there is no true hypoblast here, there is fusion only between epiblast and mesoblast. Drawn with camera,  $\times 55$ .

FIG. 18.—A diagram to show the relation of the neural plate to the "blastopore" and rest of the primitive streak. The dotted area represents the neural plate. The shaded area  $BR'$  represents the primitive streak at the time just prior to the commencement of the folding up of the neural plate. The space  $BR$  between the two concentric circles represents the original position of  $BR'$ . The asterisks denote two points in the epiblast which will come together when the neural plate has folded up, and the daggers denote two other points alongside the blastoporic lips which will come together as explained in the text.

FIG. 19.—A diagram to show the relation of the neural plate to the blastopore and rest of the primitive streak, and to show the way in which a portion of primitive streak becomes folded up along with the neural folds. It will be seen that here the folding is nearly completed; the asterisks and daggers of



Fig. 18 have been brought together so as to almost coincide. This figure also diagrammatically represents the anus as a reopening of the ventral part of the original blastopore. This diagram and the diagram of Fig. 18 represent nearly the stages of the drawings Fig. 23 and Fig. 11 respectively.

FIG. 20.— $4\frac{1}{2}$  mm. Tadpole. A horizontal section through the posterior end at a level corresponding to that of Fig. 15, but of an older embryo. Instead of the layers being fused along the middle line, they are now separated and distinct. Camera drawing,  $\times 55$ .

FIG. 21.— $4\frac{1}{2}$  mm. Tadpole. A horizontal section through the posterior end at a level corresponding to that of Fig. 16, that is to say, through the anus, which is now completely formed. Camera drawing,  $\times 55$ .

FIG. 22.— $4\frac{1}{2}$  mm. Tadpole. A horizontal section through the posterior end at a level corresponding to that of Fig. 17. These three sections, Figs. 20, 21, and 22, are from the same series. The sections are not stained, and the drawings have been made to represent the actual shades of the three layers as nearly as possible. Camera drawing,  $\times 55$ .

FIG. 23.— $2\frac{1}{2}$  mm. Tadpole. A surface view of the posterior end of a tadpole, in which the neural folds have met and are fusing. The "blastopore" is still open, and the anus is conspicuous. The horizontal lines numbered 14, 15, 16, and 17 are drawn at the levels at which the sections were taken, from which Figs. 14, 15, 16, and 17 were drawn. The bracket, *PS*, includes the dorsal and ventral limits of the primitive streak. This figure was drawn partly from a specimen hardened in Kleinenberg's picro-sulphuric acid, and partly from a model constructed by pasting together in order pieces of cardboard corresponding to a complete series of camera drawings.

FIG. 24.—A sagittal section of the posterior three quarters of a frog embryo, in which the neural plate is beginning to fold up, and in which the anus of Rusconi has become much reduced. The yolk-plug has been retracted, and the embryo is lengthening. The extent of the primitive streak is indicated by the bracket *PS*, and by the diagonal shading.

FIG. 25.— $2\frac{1}{2}$  mm. Tadpole. A semi-diagrammatic sagittal section through the posterior half of a frog embryo, of the same age as that from which Fig. 23 was drawn. The extent of the primitive streak is indicated by the bracket *PS*, and by the diagonal shading. The neural plate has completely folded over, but has not yet separated from the external epiblast. This figure shows the dorsal portion of the primitive streak folded over with the neural plate, and with it about to be separated from the adjoining epiblast. The blastopore has not quite closed in the middle line. The neurenteric canal is seen to be formed of two portions; the ventral, being the original opening from archenteron to exterior, the blastoporic canal (*B. C.*) or blastopore, and a dorsal, the canal through the folded-up lateral lips of the blastopore, or

portion of the primitive streak ( $PSO$ ). The anus has not yet completely perforated the ventral part of the primitive streak.

FIG. 26.— $4\frac{1}{2}$  mm. Tadpole. A semi-diagrammatic sagittal section through the posterior end of an embryo in which the tail has just begun to grow out. The central nervous system has entirely separated from the skin, and with it that portion of the primitive streak which was folded up with it ( $PS''$ ). By this means that portion of the primitive streak ( $PS''$ ) comes to lie now within the embryo. The ventral moiety of the primitive streak has ceased to exist as such, and the portions derived therefrom are shown by cross-shading; while the dorsal portion, or still functional portion, if it may be so termed, is indicated as in the two immediately preceding figures by diagonal shading. The bracket  $PS$  includes the full extent of primitive streak or its derivatives.

On some Points in the Histology and Development of *Myriothela phrygia*.

By

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With Plates XXXVI and XXXVII.

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WHILE working at the Marine Biological Laboratory at Plymouth during the summer of 1888 my attention was directed to the remarkable hydroid *Myriothela phrygia*. I was acquainted with Professor Allmann's (1) memoir, and it appeared to me that there were several points which demanded further study—notably the development of the gonophore and the structure of the endoderm, especially in relation to the physiological processes taking place in the enteric cavity. My work was continued, so far as circumstances would permit, during the ensuing winter; but on the appearance of Korotneff's account (2) of the growth of the gonophore, published in the 'Archives de Zoologie Expérimentale' for that year (1888), I discontinued it, in the hope that at some future time I might find the opportunity of examining with something like completeness the physiology of *Myriothela*. That opportunity unfortunately has not arisen, and I am induced to publish my results as they stand, believing that though incomplete they contain certain points of interest.

The first complete account of the anatomy and development of

Myriothela is that of Professor Allmann, which was published in the 'Phil. Transactions' in 1875. This was followed by a long monograph illustrated with abundant figures, and published at Moscow (3) in 1880, unfortunately in Russian, by Korotneff, who was the first to study Myriothela with the aid of properly prepared sections.

In 1881 Korotneff published a further paper dealing with the same subject (4). A copy of this I have unfortunately not been able to obtain. The further literature of the subject will be referred to as occasion demands in the following pages.

The work was carried out partly in the Marine Biological Laboratory at Plymouth, and I am grateful for the many kindnesses experienced there. But the bulk of the work was done in the Morphological Laboratory of Cambridge University, and I would here thank Mr. Sedgwick for placing the resources of his department at my service.

The general structure of Myriothela will be best learned by reference to Allmann's monograph, and to that I must refer my readers, merely stating here that it is a solitary attached hydranth. The proximal part of the body is usually bent at right angles to the rest, is covered with a thick perisarc, and gives origin to short processes by which it is attached to the under side of large stones. The perisarc of the foot is represented over the rest of the body by a delicate cuticle. Following on the foot is the middle region of the body, whence spring numerous blastostyles. Each of these bears gonophores, male and female, on its proximal portion, while short capitate tentacles spring from the distal extremity. The blastostyles are without a mouth.

The distal or oral region of the body of the hydranth is the longest, and is studded with very numerous, small, capitate tentacles to within a short distance from the mouth.



THE EARLY STAGES IN THE FORMATION OF THE GONOPHORE  
AND THE ORIGIN OF THE SEXUAL ELEMENTS.

The points noticed under this heading may be regarded as an addendum to Korotneff's account of the development of the gonophore (3) alluded to above. His brief and somewhat diagrammatic account is illustrated by figures which unfortunately show little attempt at histological detail.

The Structure of the Ectoderm of the Blastostyle.—In each blastostyle two regions may be distinguished; a distal and shorter region bearing several small capitate tentacles, and a proximal region, on the whole free from tentacles, on which the gonophores are developed. The latter embraces two-thirds to four-fifths of the whole length of the blastostyle. The ectoderm of the distal portion resembles that of the body generally, with the exception that the muscle-fibres, which form its deepest layer, are not so strongly developed. Its structure is shown in Pl. XXXVI, fig. 1. Like the ectoderm of the animal generally, it is covered superficially by a well-marked cuticle, which, when stripped off and examined with a high power, is seen to be divided into irregular areas, doubtless corresponding to the subjacent cell layer. This latter is composed of long columnar cells, tapering at their lower end. Each cell is about  $25\ \mu$  long, though their dimensions possibly vary according as the whole blastostyle is in a condition of extension or contraction. Lying here and there between the pointed bases of these cells are scattered ganglion-cells, which are connected with a rich plexus of nerve-fibrils situated in the deepest part of the ectoderm and immediately over the muscle-fibres. The latter run longitudinally, and immediately overlie the supporting lamella. Fibrils from the basal nerve plexus pass between the columnar cells towards the surface, and, in tangential sections, are seen to form a superficial plexus between the columnar cells.

The same type of nucleus is found throughout the whole ectoderm. It is characterised by the presence of a distinct

nucleolus linked by scattered fibrils with irregular patches of chromatin on their course to a deeply-staining envelope.

The cell-substance of the columnar cells is granular and turbid, the granulation mostly being fine. Picro-carmin or hæmatoxylin stains it only slightly. The ganglion-cells, on the other hand, stain well with picro-carmin.

The ectoderm of the proximal or gonophore-bearing region is vastly different from that just described. In the first place it is much thicker and more complex, being composed of more varied elements. The ectoderm of the distal region is about 30 to 35  $\mu$  thick, of the body 40 to 50  $\mu$ , while that of the gonophore-bearing region varies from 50  $\mu$  to 70  $\mu$  in thickness. The only other region in which the ectoderm at all approaches it in thickness is in the foot. To this fact we will return later.

The second striking feature of the proximal ectoderm is that its characters are not constant. It is most complex and thickest in specimens killed in spring and early summer, while in autumn it is not only much thinner (30 to 35  $\mu$ ), but also presents the appearance of being exhausted. A comparison of fig. 2 with fig. 4 will render this abundantly evident. The following description applies to specimens killed in March, April, and May.

Starting from the outside we have first a well-developed cuticle, which overlies cells resembling the columnar cells of the distal region, but, for the most part, shorter and broader. They are composed of the same ill-staining granular protoplasm, and the border between cell and cell is often so indistinct that we might almost call this with Allmann a nucleated protoplasmic layer. The proximal region is abundantly armed with nematocysts, which, as was pointed out by Allmann, are of two kinds. Here and there these may be seen wedged between the columnar cells. The next following layer is a fairly distinct one of large rounded cells engaged in the manufacture of nematocysts. The protoplasm of these cells, when stained with osmic acid, appears granular under moderate magnification. With high powers (Zeiss  $\frac{1}{15}$ th ob.)

this is seen to be due to the presence of numberless minute vacuoles. Each cell has embedded in its substance a hyaline mass of uniform texture (fig. 2), which at first is deeply placed in the cell, but as growth proceeds it is pushed to one side (fig. 5 *b*), and eventually forms one of the curious rhabdite-like nematocysts which stain so deeply with hæmatoxylin and osmic acid. The first formed amorphous hyaline masses, however, stain only lightly with osmic acid, and, whatever the chemical change may be which alters so profoundly their behaviour towards that reagent, it does not occur until a relatively late period in their development. I am not at all certain that the hyaline masses found in these cells are, in every case, early stages in the development of nematocysts. For many reasons I am inclined to think that they may sometimes be of the nature of reserve nutritive material. My researches on this point are, however, incomplete, and I will merely content myself here with noting the fact that the very earliest change in that part of the ectoderm where a gonophore is about to be developed, is amongst other things a local accumulation of the large cells bearing hyaline masses, sometimes one, sometimes two (fig. 8), while at the same time there is a total disappearance of nematocysts from that area.

The deepest layer of the ectoderm of the gonophore-bearing region is the most remarkable. It consists for the most part of small rounded cells (fig. 6), either scattered about fairly evenly, or, and more frequently, gathered into smaller or larger clusters. If one of these clusters be examined with a high power, the cells composing it are seen to present considerable differences in size, varying from  $8\ \mu$  to  $12\ \mu$  in diameter. In most of the clusters, and more especially if the blastostyle under examination be one which has scarcely commenced to produce gonophores, few or many of the cells will be found with two small nuclei, and the cluster will betray other evidence of the active proliferation of its constituents (fig. 2).

In those cells with a single nucleus, that nucleus is uniformly of about  $4\ \mu$  in diameter, and, like the nuclei generally, is



characterised by the possession of an exceedingly distinct nucleolus. The smallest of these cells are generally isolated (fig. 6), and each consists of a nucleus surrounded by a delicate pellicle of exceedingly finely granular protoplasm. The whole cell may be only  $5\ \mu$  in diameter. They form the distinctive feature of the ectoderm of the proximal region of the blastostyles, and, by their number, give to it its great thickness. What part they play we shall see later.

The remaining constituents of the proximal ectoderm as yet unnoticed are its nervous and muscular elements. These I propose to mention very briefly, for they lie to a certain extent outside the limits of the present paper. One of the most striking features of osmic acid preparations, whether sections or teased, are tufts of branching filaments with curious deeply-staining matter disposed on them in irregular patches and granules (fig. 3). In teased preparations these filaments are seen to largely end in a thick plexus between the columnar cells (fig. 3), but some of them also end in the cells which are developing or have developed a nematocyst (fig. 5). Not infrequently a filament may be seen having on its course a mass of granular protoplasm containing a nucleus (fig. 2). Traced downwards these filaments appear to be connected with a deeply-placed nerve network,<sup>1</sup> which in turn is in close relation to the muscle-fibres which are placed immediately upon the supporting lamella.

If the ectoderm of the gonophore-bearing region of a specimen killed in the autumn be examined, it will be found to consist of externally a cuticle with columnar cells underlying it, and then an indefinite number of ganglion-cells and cells

<sup>1</sup> Fig. 5 is an accurate drawing made with the aid of a camera lucida of a portion of this nerve complex, which by good fortune was isolated in a teased osmic acid preparation. It exhibits a remarkable fact in the arrangement of this primitive nervous system, namely, that some of the ganglion-cells are enclosed in a fine reticulum, formed by the breaking up of filaments derived from the general nerve network. Since this drawing was made the remarkable researches of Golgi, Ramon y Cayal, Kölliker, and others have demonstrated the existence of similar structures in the central nervous system of the higher animals.



concerned in the manufacture of nematocysts (fig. 4). In some sections scanty patches of small cells similar to those described above may be seen, but they are now the exception and not the rule. On the other hand, over considerable regions the ectoderm may be even more decrepit than that described above, for even the columnar cell layer may be imperfect, while the basal cells are represented largely by irregular spaces. On the other hand, the muscular layer and supporting lamella are proportionately better developed. Measured from the external surface of the cuticle to the external surface of the supporting lamella the ectoderm at this period is from 30 to 35  $\mu$  in thickness.

We thus see that as the season advances and the reproductive period comes to a close the ectoderm of the gonophore-bearing region becomes more and more exhausted of those small cells which are such a characteristic feature of it in the spring, and I think there can be little doubt that their disappearance is connected with the active formation of gonophores during the summer months.

So far I have spoken of them merely as the small cells of the proximal ectoderm. It is necessary to see whether these cells are all alike, or whether they may be divided into one or more sets differing in their function and connections. In tracing the process of formation of the nematocysts I have said that they first appear as a rounded hyaline mass embedded in the protoplasm of a cell which then lies in the deeper part of the ectoderm.

The smallest cells containing these masses may be only 10  $\mu$  in diameter; but though they do not differ markedly from the bulk of the small cells in point of size, they do differ in one very important particular, namely, in the fact that they are always connected by a delicate process with the nerve network (fig. 5).

On the other hand, we have in addition to these small cells which are in connection with the nerve network, and so often contain some trace of a developing nematocyst, other still smaller rounded cells, which appear, even with the highest

powers, to be entirely free ; and these are the elements which occur so characteristically in little groups, the cells of which so frequently betray signs of active proliferation. These histological facts, together with the absence of these free cells in other parts of the body and their peculiar relation to the gonophores, entitle us, I think, to regard them as preformed sexual elements.

### The Earliest Stages in the Formation of the Gonophore and its Relation to the Process of Budding in *Myriothela*.

In early spring, and before sexual reproduction has taken place to any marked extent, specimens of *Myriothela* may be found which bear buds in various stages (Pl. XXXVII, fig. 13). These appear to be always developed just at the junction of stolon and body. Once only have I met with a bud formed elsewhere, namely, in the lower tentacular region. This had, however, more the appearance of a permanent growth than of a bud to be cast off.

The process of budding, so far as I have followed it, is a rather remarkable one. The first stage is a modification of the character of the ectoderm, which in the stolon and lower part of the body is composed of very long columnar cells, resembling the columnar cells of the blastostylar ectoderm in all particulars save in their inordinate length. Lying between the bases of these columnar cells are interstitial cells, characterised by the fact that they stain more deeply with picrocarmine. These cells appear to be partly nervous and partly concerned in the formation of nematocysts, which, curiously enough, are produced in limited number even under the thick and dense perisarc of the upper part of the foot. Where a bud is about to be formed the ectoderm-cells lose their defined characters, proliferate, and a bulging mass of amorphous tissue results. At the same time the thick supporting lamella becomes absorbed, and the endoderm-cells likewise proliferate and take on an amorphous character. The result is a kind of blastema in which the limits of ectoderm and

endoderm are undistinguishable. This grows while at the same time its elements lose their distinctness and become highly charged with spherical masses of stored nutriment, resembling in many particulars the nutritive spheres of the general endoderm. As it grows it pushes the perisarc before it, and ultimately forms a rounded egg-like mass attached to the parent body by a short thick pedicle (fig. 13). From this the young *Myriothela* is developed (fig. 13). All connection with the body of the parent is lost at a very early period, almost before the bud has re-formed its ectoderm and endoderm and enteric cavity. It remains attached to the perisarc, however, by a sucker-like arrangement at the aboral pole until it is fully formed.

As will be seen, the formation of a gonophore is, in its earliest stages, essentially similar to this method of budding. In other words, the gonophore is a true bud which, like the other buds, is derived from a blastema formed by a fusion of ectodermal and endodermal elements. The difference, however, lies in the fact that in the case of the gonophore bud, after it is a well-formed structure, a group of the primitive germ-cells make their way into it.

The first stage in the growth of a gonophore is shown in fig. 8. The ectoderm of the gonophore-bearing region becomes thickened over a small surface, the increase in thickness being due largely to an accumulation of the primitive germ-cells, but partly to an increase in the cells carrying hyaline masses. At the same time nematocysts disappear in that region of the ectoderm, though they may occur in their usual profusion in close proximity. In the next stage the basement membrane is absorbed or ruptured (figs. 7 and 9), I cannot determine which, and a tongue of endoderm-cells pushes its way into the ectoderm and through the deepest layer. Thus the cluster of primitive germ-cells come to lie not on its apex, but, generally, asymmetrically disposed on one side.

The removal of the supporting lamella is, I am inclined to think, mainly a process of solution, since scattered rounded



fragments may sometimes be seen; and further because the muscular elements are absorbed for some little space about the point where the rupture takes place (figs. 7 to 10).

The tongue of endoderm-cells rapidly becomes a tubular outgrowth, and the cells at its apex lose their nutritive spheres and become small, dense, on the whole ill-staining cells.

At this stage it is perhaps impossible to distinguish the limit between ectoderm and endoderm, while at the same time a fusion of cell substance has taken place (fig. 10), so that we have a stage closely resembling the blastema which gives rise to the bud as described above.

Fig. 10 represents this stage, and is an accurate drawing, made with the aid of a camera lucida, of a preparation from a specimen killed with corrosive sublimate and stained with picro-carmine; the whole specimen being remarkable for the good preservation and clear definition of its histological elements. The section figured passes rather obliquely through the young gonophore, but the next in the series shows that the primitive germ-cells have now travelled in under the superficial columnar cells of the ectoderm to form a cap for the fused ectoderm and endoderm.

The next stage to be noticed is in many respects remarkable. By a fresh formation of supporting lamella the whole bud with its contained germ-cells becomes separated from the maternal tissue, while at the same time a fold of supporting lamella becomes formed which separates the ectodermal elements with the primitive germ-cells from what is usually known as the endoderm lamella (fig. 11). The endoderm lamella, therefore, from this time onwards, is separated from the endoderm of the parent by a well-defined and permanent supporting lamella; and it is noticeable that up to this stage and until they degenerate the cells of the endoderm lamella are not of the ordinary endoderm type, but resemble in every detail the ectodermal cells of the gonophore.<sup>1</sup>

<sup>1</sup> The columnar cells of the maternal ectoderm remain undisturbed and unaltered by these various changes. I regard them as belonging to the



In the further history of the gonophore I am only concerned with one point, namely, the fate of the primitive germ-cells. I entirely agree with Allmann that in their earliest history it is impossible to distinguish between male and female gonophores. Position does not help one, for, as has been recognised before, both male and female elements are produced on the same blastostyle, and to a certain extent indiscriminately, the same transverse section frequently passing through both male and female gonophores. Very soon, however, the male gonophores become distinguished by the rapid proliferation of their generative elements.

In the female gonophore at some period, often relatively late, two or three of the generative cells become larger and more prominent than the others. The period at which this happens does not appear to be fixed; and whatever factor it may be, whether something inherent or accidental, that determines which of these struggling cells shall obtain the mastery and eat up its fellows, it sometimes does not come into play until the gonophore has become a well-formed structure. But it is quite late in the history of the gonophore, when that structure is large and already swollen with yolk, before these two, three or four cells, which, so to speak, have succeeded in attaining to the final heat, decide who is the winner.

In the facts which have been set down above with regard to the structure of the ectoderm of the blastostyles, and the formation of the gonophores, two main points appear to me to be of special significance. These are (1) that the gonophore appears to be a curiously modified bud, and (2) that the generative elements pre-exist as free cells having lodgment in the tissues of the adult, and only travel into the abortive bud, which is their place of final development.

maternal tissues, and not to the gonophore bud. They are, therefore, not included in the above statement.

ON CERTAIN POINTS IN THE STRUCTURE AND FUNCTIONS OF  
THE ENDODERM OF MYRIOTHELA.

In any animal three main problems concerning the manipulation of its food-stuffs present themselves. These are (1) the disintegration and solution of its food; (2) the absorption of the dissolved or liberated and unchanged material; and (3) the distribution of the products of digestion. We might also add to these the storage of prepared food-stuff. Many and diverse reasons justify the statement that a cell is hampered or, better, limited in the range of its activity by being loaded with indifferent reserve nutriment; and it therefore becomes almost the duty of a special tissue to store material, either as a provision for some extraordinary metabolic effort, or as a consequence of an infrequent and uncertain food-supply. In the case of *Myriothele* we shall see that this last problem—the storage of reserved nutriment—occupies a large part of the endoderm.

The disintegration of the food in all animals not possessed of a masticatory apparatus is a process of solution differing only in degree from the final solution of the smaller particles. Yet I think we are justified in speaking of the whole act as a process of disintegration and solution, because of the very general tendency of animals to divide the process into those two stages, and to differentiate the alimentary tract into a special region where disintegration of the food by solution takes place, e. g. the stomach of Vertebrates, and another tract where solution is completed, and where solution and absorption go on hand in hand.

In an animal of any considerable size the distribution of the products of digestion becomes a problem of the utmost importance, not only physiologically, but also from its far-reaching influence on the morphological characters of animals. It has been solved in three main ways:

1. By the utilisation of the enteric space itself, which functions in part as a digestive cavity, and in part as a common

space in close communication with all parts of the organism, and containing not only the results of the solution of the food, but also material discharged from the lining cells of one region, and destined for the nutrition of other parts of the organism. It is as an example of this class that I wish to consider *Myriothela*.

2. By the development of a system of spaces or a common space round the gut, into which the results of digestion can be discharged, and from which the tissues can directly derive their nutriment. Such a space would be the hæmocœl of morphologists. The physiological significance of the cœlom is still, I think, very much under judgment.

3. By the aid of a closed vascular system. The true relations of hæmocœl and cœlome to one another and the relations of both to the vascular system of Annelids, which appears to be initially respiratory; and to the vascular system of Arthropods, which is in its first inception a mechanism for the circulation of the fluids of the circum-enteric space, are questions which do not concern us here, but the interrelation of cases 1 and 2 demands brief notice.

It is necessary at the outset to distinguish clearly between the digestive functions of the enteric space, and the part it may play in the distribution of the nutritive material. The researches of Miss Greenwood on the digestive process in *Hydra* justify the conclusion that in that animal the enteric space is used mainly, if not solely, for digestion. The endoderm-cells forming its walls absorb and largely store the products of digestion. There our exact knowledge ends, but the uniform character of the endoderm throughout the entire animal, the fact that its cells everywhere, even in the tentacles, absorb and store nutriment, renders it probable that in the physiology of *Hydra* the discharge of elaborated nutritive material into the enteric space to form a common nutritive fluid akin to the blood of higher animals plays little part. But in the higher Cœlenterates, in the colonial forms, in *Medusæ*, and in *Ctenophora* especially, we have no reason to doubt that such a fluid does exist, and that it forms the metabolic link between the

different regions of those animals in such a way that the demands of one part may be met by the discharge of stored nutritive material from other regions. Such a fluid, which Allmann has called the "somatic fluid," would not only contain the immediate results of digestion, but also elaborated material from the store of reserve nutriment possessed by certain cells discharged in response to any special demand in some particular region. In other words, it is not merely a fluid for the distribution of the immediate products of digestion; it is more than that, and we shall see reason to think that it is a true metabolic link between one part of the body and another strictly comparable to the blood of higher forms.

In Allmann's account of digestion in Hydroids he thus describes the "somatic fluid:"—"Its basis is a transparent colourless liquid, and in this solid bodies of various kinds are suspended. These consist partly of disintegrated elements of the food; partly of solid coloured matter which has been secreted by the walls of the somatic cavity; partly of cells, some of which have undoubtedly been detached from those walls, though it is possible that others may have been primarily developed in the fluid; and partly of minute irregular corpuscles, which are possibly some of the effete elements of the tissues."

Leaving the Cœlenterates and turning to the Turbellaria, we have a striking instance of how the enteric cavity, the gut, may serve as an organ for the distribution of nutriment. The case of the Turbellaria also enables us to contrast cases 1 and 2, for in the Rhabdocœls we have animals with a simple gut surrounded by a tissue, the mesenchyme, with numerous cleft-like spaces, which may be so far developed as to form a fairly well-marked space round the gut, as in *Mesostoma tetragonum*, and to a less extent in *Microstomum lineare*. That such a space or system of spaces facilitates the distribution of material derived from the gut hardly needs stating, whether we consider the distribution to be brought about by diffusion or by the agitation of the contained fluid as a result of the muscular movements of the animal.



In the Triclad and Polyclads, on the other hand, the same problem has been solved in a rather different fashion. The development of spaces round the gut is not the most obvious fact, but the gut itself ramifies into a network of tubes which penetrate to every part of the body.

The Structure of the Endoderm. — Leaving these general considerations, we will pass at once to a consideration of the structure of the endoderm in *Myriothela*.

The mouth is bounded by a thin membranous lip, or hypostome, which is exceedingly muscular and sensitive, and is probably of considerable use in seizing the prey. It is figured by Allmann and Hincks in a condition of extension. In fig. 20 it is shown as it appears when the animal is retracted.

In the lip the supporting lamella is either reduced to the thinnest film or is entirely absent. It is always absent from about half the breadth of the lip from the free edge. The ectoderm of the external surface is rich in nerve-cells, and overlies a well-developed layer of radial muscles. At the free edge there is no loss of histological continuity, the ectoderm being continued on to its under surface for a certain distance, and having the same structure. Passing downwards towards the attachment of the lip, there appears at the base of this ectodermal epithelium a more and more defined layer of highly vacuolate cells, which are the first commencement of the endoderm. In other words, the ectoderm for a short distance overgrows the endoderm, thus forming a distinct zone of mixed character.

At the point where the lip merges into the tentacle-bearing region the ectoderm, as a distinct structure, has finally disappeared, and we have the arrangement shown in fig. 14. Three kinds of cells may be distinguished: (1) a superficial layer of elongated cells, staining well and uniformly with picro-carmin. Each possesses a nucleus and nucleolus in its basal portion, which is tapering and wedged in between the subjacent cells. In favorable preparations these cells appear covered with short, fine cilia. These are, however, not always visible, mainly because the animal in dying usually

retracts this region to such an extent that the epithelium is thrown into deep folds, and the free surfaces of the cells apposed. This ciliated zone forms only a narrow band. Between the bases of the ciliated cells are (2) numerous rounded and deeply-staining cells, which are more numerous the nearer the lip. Delicate processes may be sometimes seen passing from them to the surface, and they are probably sense-cells. Below these, and forming by far the greater portion of the epithelium, are strikingly characteristic palisade-like cells. Each has a very scanty and ill-staining protoplasm, which surrounds a large irregular vacuole occupying the bulk of the cell. Two nuclei, each with a small nucleolus, are usually present, and may be either close together about the middle of the cell, or one at either end. There is not the slightest evidence that the presence of these twin nuclei indicates cell division.

At the lower edge of the ciliated zone conical cells appear between and rapidly replace the ciliated cells, while at the same time the deep-staining sense-cells disappear. Each of these conical cells resembles in its general appearance a goblet-cell of an ordinary mucous membrane. We can, therefore, conveniently style the next region the goblet-cell zone. This embraces a considerable portion of the tentacle-bearing region. It is, however, impossible to reduce the relative dimensions of these various zones to numerical exactness because of the extreme extensibility of this portion of the animal.

Each goblet-cell is, as its name implies, flask-shaped, and consists of an expanded part which stains lightly with picro-carmin (fig. 16) or osmic acid (fig. 15). The contents of this part are turbid from the presence of ill-defined, granular masses. The expanded portion of the flask is continued downwards into a tail, which contains a small nucleus embedded in deeply-staining granular protoplasm. The numerous and coarse granules of the basal portion stain deeply with osmic acid, and less so with picro-carmin. These cells are undoubtedly of the nature of gland-cells, and the well-formed basal granules may be regarded as the first stage in

the elaboration of the secretion product which occupies the expanded portion of the cell. In the upper part of the goblet-cell zone the endoderm is composed of goblet-cells lying wedged between the apices of palisade-like cells exactly resembling those occurring in the ciliated zone, except in the fact that they now abut on the free surface. When this portion is retracted the deep folds present the appearance in sagittal sections of long tubular glands, which, from the character of the abundant goblet-cells, closely simulate the crypts of the large intestine.

In the middle and lower part of this region the endoderm is thrown up into low conical villi. These structures are characteristic of the whole of the endoderm with the exception of the part already described, that is in the neighbourhood of the mouth, and in the foot. They vary very much in length in the different regions of the animal, but are usually longest in the lower portion of the tentacle-bearing region, where they are long filiform structures, sometimes branched, and may measure .3 to .5 mm. from base to apex. Generally speaking, the villi are not muscular, but in the goblet-cell region they appear to have a distinct muscular axis.

#### Structure of a Villus from the Goblet-cell Zone.

A section through the axis of one of these is shown in fig. 16. On the sides of each villus, and between their bases, we have the same arrangement of palisade-cells and goblet-cells as that described above. At the apex, however, are a group of cells presenting many new features. These I propose to call the apical cells, and they form not only the apex of the villus, but are also continued downwards as its muscular axis.

Each apical cell has the following general structure. The protoplasm is abundant, and stains deeply, thus offering a marked contrast to the other cells of the villus. It is also turbid and opaque, but differs very much in this and in its behaviour with stains in different parts of the cell. In the muscular stem, however, the protoplasm is always



of a uniform texture, and behaves towards osmic acid and other stains in a manner closely resembling the muscular elements of the ectoderm. The protoplasm of the expanded end of the cell encloses the following structures. One, rarely two, nuclei, which vary so much in position as to suggest an extreme mobility of the contents of the cell. A varying amount of pigment, dark brown in colour, and disposed in scattered grains near and on the free surface of the cell, but usually in little heaps of grains in the deeper parts. One or more large vacuoles. And, lastly, turbid masses of substance, some of which are certainly the remains of material which has been ingested by the cell, and all of which are in more or less obvious relation to the vacuoles.

These factors sum up the constituents of the apical cell as usually seen; but the extent and character of the vacuoles and the turbid masses of enclosed matter vary very much at different periods and in adjacent cells. To this, however, we will return later.

In the varying position of the nuclei we have some indication of the mobility of the protoplasm of the apical cells; and this mobility finds further expression in the fact that from the free surface of the cells pseudopodial extensions are pushed out, especially from those cells which are fairly free from enclosed masses (cf. Allmann's 'Memoir,' pl. lvi, fig. 2).

In the middle tentacular region the endoderm assumes a different character. The goblet-cells disappear, and the palisade-cells gradually pass into a shorter and broader type of cell, mostly with only one nucleus. These cells I will call vacuolate cells, adopting the term applied by previous observers to similar cells occurring in *Hydra*. These cells usually contain numerous round hyaline corpuscles, which vary in their characters but stain always with osmic acid and many aniline dyes (such as methyl blue and green), but typically take no coloration with hæmatoxylin and little or none with carmine stains. These are, without doubt, identical with the sphere-like masses of reserve nutriment described by Miss Greenwood as occurring in the vacuolate cells of *Hydra*.



under the name of "nutritive spheres." The vacuolate cells of *Myriothele* when free from nutritive spheres may be seen to possess a large vacuole surrounded by scanty protoplasm. In this condition they recall the palisade-cells of the oral region, and the intermediate types are so numerous that I am disposed to regard these cells as fundamentally the same. The most constant differences are that the palisade-cells have almost always twin nuclei, and only rarely contain nutritive spheres.

Wedge-shaped here and there between the vacuolate cells are other and smaller dark-staining cells (fig. 21), which occupy the same position but are not disposed with the same regularity as the goblet-cells. These cells are as variable in size and appearance as the vacuolate cells, and they correspond to the "gland-cells" of Nussbaum and Jickeli. Miss Greenwood has shown reasons for considering that cells similar to these occurring in the endoderm of *Hydra* are concerned in the formation of the digestive enzymes, and I find that her conclusions are warranted by the different appearances presented by these cells under varying conditions in *Myriothele*. To this point we will return later.

Rarely a gland-cell may be seen apparently bearing a delicate pseudopodium or flagellum, and having the appearance shown in fig. 18*b*. Nussbaum similarly describes cilia on the gland-cells of *Hydra*.

These gland-cells are very widely dispersed throughout the endoderm. They occur, perhaps, in greatest abundance on the sides of the villi; sometimes, however, one or two may occur at the apex of a villus. Rarely, at the apex of a villus, a group of two, three, or four small cubical darkly-staining cells is found (fig. 19). Whether these are or are not stages in the development or multiplication of gland-cells I was unable to determine. That they are, however, the antecedents of the free corpuscles which at certain periods occur in the somatic fluid I see no reason to doubt.

The gland-cells of *Myriothele* have a rather wider distribution than those of *Hydra*, where they are restricted to the

body. In *Myriothela* they occur in their greatest abundance in the endoderm of the lower half of the tentacle-bearing region. But they are also numerous in the middle region of the body whence the blastostyles spring, and may even occur in limited numbers in the endoderm of those structures near their points of attachment.

In the middle and lower regions of the body the endoderm is to a certain extent different from that already described. The villi, as a rule, become less muscular, while at the same time their apical cells change their characters and become more and more akin to the vacuolate cells. The endoderm of the body-wall from which the villi spring, and which in the lower tentacle-bearing region is composed of cells in no wise distinguishable from those lining the villi, changes its character in the blastostyle-bearing region. There it is composed of long columnar cells, each with a single nucleus, and each composed of dense well-staining protoplasm free from vacuoles.

The endoderm maintains these characters in the foot; that is to say, the villi, which here are of the nature of broad flange-like folds, are covered by vacuolate cells with rarely a gland-cell, while the endoderm of the body-wall is composed of the deeply-staining columnar cells. At the extreme end of the animal, however, the supporting lamella ceases to exist, and to a certain extent the limits between ectoderm and endoderm become obscured, and a kind of growing-point to the creeping stolon is the result.

The endoderm of the blastostyles will receive special mention later. To make this general account of the whole endoderm complete, however, I will merely state here that the villi in the blastostyles are low conical structures almost exclusively composed of vacuolate cells. A few gland-cells lie scattered in the proximal third of each blastostyle.

Taking the most general view of the structure of the epithelium lining the enteric space of *Myriothela* as described in the preceding pages, we see that it may be divided into different regions. These are—(1) An oral region characterised by the

presence of sense-cells and cilia in its upper part, and of numerous glandular cells, the goblet-cells, in its lower part. (2) A middle zone comprising the middle region of the entire animal, and characterised by the presence of numerous gland-cells. (3) The blastostyles and the foot region, where the endoderm is almost exclusively composed of vacuolate cells, usually loaded to the full with stored nutritive material in the form of nutritive spheres.

Of the function of the goblet-cells I can say little. From their position I had supposed that their stored material was discharged when the food was first received, and that they were concerned in the elaboration of a digestive ferment or ferments. But I have found them apparently unaltered in animals which have just taken in their prey (a crustacean). The glairy, sticky appearance of the contents of the expanded portion of the goblet, however, suggests the idea that they form a strongly adhesive surface to what may be called the prehensile portion of the endoderm. The gland-cells, on the other hand, present no especial difficulties. Fig. 18*a* represents one shrunken and discharged as seen in an animal at the close of a digestive act, that is with merely the detritus of a meal in its enteric cavity. Fig. 18 shows one taken from a fasting animal. It is fully loaded with granules. Fig. 18*b* represents an intermediate condition. The granules are large and coarse, and appear to be formed in the deeper portions of the cell. Their discharge is characteristic, and may be witnessed in preparations from an animal which has just ingested its prey. The granules are extruded, apparently unchanged, into the somatic fluid, there to be dissolved. That is to say, they do not break down to form the digestive enzymes until they are free from the cell in which they were formed (fig. 21).

The Process of Digestion.—For a long time I was unable to determine the natural food of *Myriothela*, while at the same time all endeavours to induce it to ingest pieces of raw meat or fragments of Molluscs and Crustacea completely failed. I therefore, in the meantime, turned my attention to carmine and sodium sulphindigotate, and with a fine pipette,



inserted through the mouth, injected a drop of sea water containing the one in suspension or the other in solution. Later, however, I was more fortunate, and succeeded in obtaining specimens with food in the enteric cavity. In one case the prey was a Crustacean of some considerable size, so that it produced a very obvious bulging of the animal. I did not witness the capture and ingestion of the prey, but the specimen was killed before the digestive fluid had produced any change in the tissues. Fig. 21 is taken from this specimen. It also contained the remains of a previous meal in the lower part of the enteric space. Other specimens furnished other stages, and in this way, as a result of an examination of a large number of animals, I was enabled to obtain a series representing, to a certain extent, the various stages of digestion.

*Myriothela* is carnivorous, and captures small Crustacea. In one case the meal consisted of a half-digested egg, either derived from the gonophores of the individual in question, or from those of its neighbours.<sup>1</sup>

Digestion is carried on at first in the lower portion of the tentacle-bearing region—that is, in that region where the gland-cells are most abundant, and results in a disintegration of the prey, brought about by the agency of the digestive fluid.

The gland-cells which first discharge their contents are those in the immediate neighbourhood of the meal, but even there all the cells are not affected at once, those which are most loaded with granules probably being discharged first. Some of the gland-cells in the proximal region of the blastostyles and in the foot may be found undischarged until nearly

<sup>1</sup> The huge yolk-laden eggs, when set free from the gonophores, are taken by tentacle-like bodies, the "claspers," which hold them while development proceeds and until the actinula larva is fully formed. In March and April, however, the claspers are not always present, and the eggs formed then when ripe are shed and sink to the bottom, where they become attached. I think that further research will show that these early eggs have a more direct development than those formed later, and pass at once to the form of the adult without the intervention of the free-swimming actinula stage. It was one of these free eggs which apparently had found lodgment in the enteric cavity.



the close of a digestive act. The disintegration of the prey is accompanied by a great amount of solution, so that after digestion has been in process for some time the enteric cavity contains a number of fragments of the prey floating in a fluid rich in proteids, which form a deeply-staining granular precipitate after treatment with corrosive sublimate. The disintegrated fragments of the meal find their way into the foot and blastostyles, the somatic fluid probably being circulated by the active movements of the animal, which include the extension and retraction of the blastostyles, and possibly by the cilia, which appear to be borne here and there by the endoderm-cells. The process of digestion is therefore largely extra-cellular; but this is not all, intra-cellular digestion takes place to a marked, but undoubtedly a subordinate extent, and is mainly, perhaps entirely, confined to the amœboid and mobile apical cells of the villi in the tentacular region.

If these cells be examined in an animal killed towards the close of digestion they will often be found to contain the more or less imperfect capsules of thread-cells or irregular fragments of hyaline material, which recall the cuticle of a Crustacean, and probably are fragments of that structure.

In fig. 17 such a nematocyst is shown embedded in a turbid mass of darkly-staining material, which occupies a vacuole in the cell protoplasm. At *n* is another nematocyst, now no longer lying in a vacuole, but embedded in the cell protoplasm. The digestion of the food-mass with which this nematocyst was associated having been completed, the vacuole has filled up.

Other interesting points may be mentioned which point to this intra-cellular digestion. In some cases an enclosed mass may be found embedding a more or less perfect nucleus. By a careful examination of different cells we may see such nuclei in various stages of disintegration, until they are finally resolved into chromatin granules which are scattered through a pseudopodial-like lobe of protoplasm, which appears to be thrust into a vacuole as the digestion of its contents becomes complete.

Carmine grains injected into the enteric cavity are eagerly

ingested by the apical cells, both in the body of the animal and in the proximal region of the blastostyles.

Towards the close of digestion a few free cells appear in the somatic fluid. Each is rounded and composed of dark-staining protoplasm embedding a nucleus with contained nucleolus.

Before turning to the further fate of the food, that is to say before considering the process of absorption, it will be well to describe more particularly the contents and characters of the vacuolate cells.

These when unloaded with nutritive spheres present the appearance shown in fig. 18, where the cell is seen to consist of a thin pellicle of protoplasm surrounding a large central vacuole and embedding a nucleus. The protoplasm stains only slightly. The outline of the cell is exceedingly sharply defined by some staining material which has almost the appearance of a cuticle. The process of loading with nutritive spheres is remarkable, and essentially similar to that described by Miss Greenwood as occurring in *Hydra*. The protoplasm at one point develops a small vacuole which increases in size, and bulges into the large vacuole. In this the nutritive sphere is formed from the turbid semi-fluid material which first fills it. This process continues until the whole of the cell becomes occupied by small vacuoles, each containing a nutritive sphere. The size of the cell, therefore, does not necessarily vary according to the amount of reserve nutriment it contains. This, however, only holds good for the vacuolate cells of the body. In the blastostyles they are slightly different. In the first place the large central vacuole is not developed, with the consequence that when the cell discharges its nutritive spheres it frequently shrinks to the condition of a cell with dense, non-vacuolated protoplasm, which stains deeply with picro-carmin. In fig. 22, at 1 is a cell without nutritive spheres, and at 2 one partly filled with vacuoles containing nutritive spheres.

But the discharge of the nutritive spheres does not always leave the cells smaller and solid. The vacuoles may persist (fig. 22, 4), and the result is a remarkable cell with bubbly

protoplasm. Such cells form a very striking feature of osmic acid preparations. The fluctuations in the size of the individual cells in the endoderm of the blastostyles naturally leads to a corresponding fluctuation in the total bulk of that tissue. In specimens taken in May or June it sometimes almost fills the cavity of the blastostyles.

We therefore have in the blastostylar endoderm, in addition to the scattered gland-cells, the following:—(1) Small dense cells with nucleus and nucleolus which stain deeply. (2) Cells whose protoplasm is completely occupied by vacuoles, each of which, some of which, or none of which contain nutritive spheres. And there are numerous intermediate stages between (1) and (2), with either a small or a large portion of the cell substance occupied by vacuoles. The protoplasm of cells (1) and (2), or of the intermediate stages, appears in osmic acid preparations to be remarkably dense and almost glassy, and the vacuolation is limited to the large vacuoles which embed the nutritive spheres. But other vacuolate cells occur, forming a third class, whose protoplasm is not of this character, but is so occupied by vacuoles of all sizes as to give the whole cell a very characteristic appearance (fig. 22). The vacuoles always differ in size in different parts of the cell, being larger near the free surface where they may contain young nutritive spheres. These cells, forming the third type of vacuolate cell to be seen in sections through the blastostyle, may be regarded as cells which are actively forming nutritive spheres.

Before considering the processes going on in the vacuolate cells generally, it will be well to turn to the nutritive spheres themselves. The endoderm generally contains three kinds of formed bodies—that is to say, bodies which are not of the nature of material merely ingested by the cells, but rather are new formations resulting from the activity of those cells.

Of these the most common are small spherical bodies, generally about 3  $\mu$  in diameter. These crowd the endoderm-cells in great numbers, but are always most numerous in the foot and blastostyles. They stain uniformly, but not so deeply as to become opaque, with osmic acid, and when perfect appear



singularly hyaline and structureless even with the highest powers; they do not stain at all with picro-carmin or hæmatoxylin, but take a deep tinge with aniline blue and methyl green.

They are of a complex character chemically, with probably a proteid basis. That they are not wholly proteid is, I think, shown by the fact that the coloration obtained with the xanthoproteic reaction, and with acidulated ferrocyanide of potassium and ferric chloride, is never intense though it is sufficiently distinct. Neither iodine nor iodine and sulphuric acid give distinctive results. They are also not of a fatty nature, for exposure for many hours to hot turpentine fails to materially change them. These are not only the most abundant, but the most permanent nutritive spheres.

The second class of bodies is markedly distinct from those just mentioned, and they occur sometimes in considerable abundance. They are perhaps most noticeable at the close of a digestive act. They measure as a rule  $12\ \mu$  in diameter, and are spherical bodies, each embedded in a vacuole of a vacuolate cell, which may or may not contain at the same time the small nutritive spheres. Like the latter they are sometimes homogeneous bodies showing no internal structure, and then they stain very intensely with picro-carmin (fig. 27). But they may be also found composed half of intensely staining homogeneous material, and half of more turbid material which stains scarcely at all (fig. 28). In yet other cases the intensely staining material is reduced to a small spherical nodule or patch placed excentrically on the surface of the sphere. There can be no doubt that these are various stages in the formation or destruction of the same bodies, but I do not think that the evidence at my disposal justifies me in deciding which stage is which. As a general rule, however, the smallest of these bodies, perhaps but little larger than the first-mentioned type of nutritive sphere, are homogeneous and deeply staining, while without exception the largest forms are those with the deeply-staining material reduced to an excentrically placed bleb. These bodies resemble in some respects the yolk



spherules of the ripe egg, and they are also found in some abundance in the young buds. I can throw no light either on their special significance or on their relation to the small nutritive spheres. I might finally add that they are rarely widely distributed throughout the endoderm, but usually occur in localised patches as though they were related to some localised and special metabolic process. They are perhaps never completely absent.

Though the term nutritive sphere can, I think, be applied with justice to both the preceding bodies, its application to the third class of endodermal products would be misleading, since they are merely a specialised product of the endoderm of the tentacles.

When abundant, each cell of the endoderm of a tentacle may contain one of these bodies, and then they doubtless give rise to that opacity which was noted by Allmann. They form, however, a very variable element, for while one tentacle may be fully charged with them its neighbour may contain few or none. Each of these bodies is, when fully formed,  $10\ \mu$  in diameter, and consists of a sphere which stains intensely with picro-carmin (fig. 26), and is embraced by a cup-shaped capsule with expanded edge.

That these bodies play any great part or are at all concerned in the general nutrition is extremely improbable. Under certain circumstances they are discharged from the endoderm-cells of the tentacles and find their way into the enteric space, and are ingested and digested by the apical cells of the adjacent villi.

In what I have to say concerning the formation and fate of the nutritive spheres in the following pages, I shall refer exclusively under that title to the small nutritive spheres which are so abundant and numerous. The method of formation of these bodies has been already described, and it was seen that they develop in vacuoles formed in the protoplasm of the vacuolate cells; and I see no reason to doubt, but rather every reason for agreeing with Miss Greenwood's view, that these bodies are formed from material absorbed in the fluid form

from the results of digestion in the enteric cavity. The fate of sodium sulphindigotate when introduced into the enteric cavity is interesting in this connection, for though a considerable portion of that pigment is rapidly decolourised, some may be found after a short period in the vacuolate cells associated with the nutritive spheres in the various stages of their formation in such a way that these bodies are tinged with blue in irregular patches. We may conclude, therefore, that the substances resulting from the solution of the tissue of the prey are mainly absorbed by the vacuolate cells, while at the same time ingestion of disintegrated fragments, possibly of a resistant nature, takes place to a limited extent, and is carried on by the apical cells of the villi in the middle region of the body. And the distribution of the products of digestion to the more remote portions of the endoderm is effected by the agency of the general somatic fluid.

But, as I said before, there are reasons for believing that the somatic fluid is more than a vehicle for the distribution of the immediate results of digestion. Let us now turn to a consideration of those reasons.

In the blastostyles the extensive accumulation of nutritive spheres is in obvious relation to the active and important processes carried on in those structures. But the nutritive spheres are stored in equal abundance in the endoderm of the foot, where, during the summer and autumn, there appears to be no great call for this enormous reserve of nutritive material. On the other hand, throughout the whole tentacular region and usually in the endoderm of the tentacles themselves no abundant reserve of food-stuff is present. It is, as I pointed out above, highly doubtful whether the peculiar bodies sometimes found in the endoderm of the tentacles are of the nature of simple reserve nutriment. On the other hand, it is equally certain that the actively motile and sensitive tissues of the tentacular and oral regions are not supplied directly and solely from the immediate products of digestion. We are, therefore, compelled to conclude that the nutritive material stored in one region of the body may be conveyed as occasion

demands to regions quite remote. In other words, we must suppose that the endoderm is not merely a collection of cells each fighting for a share of the nutritive material to be ultimately used by itself or by the ectoderm with which it is anatomically in immediate relation, but rather that the metabolic activities of the units of the endoderm throughout its whole length are linked together into one consistent and interdependent whole.

What is the nature of that link, and how is the interchange of material which it implies between widely separated regions brought about? That it is effected entirely by the laborious passage of material from cell to cell throughout, perhaps, a considerable length of the animal is, I think, an impossible suggestion. Yet this process undoubtedly takes place to a certain extent, and I think we may see in the palisade-cells of the oral region, from the contents of whose enormous vacuoles amorphous masses are precipitated by the action of corrosive sublimate, a mechanism for facilitating such a process, and whereby nutritive material may find its way even to the lip, a region which during the early stages of the digestion of prey of any considerable size must be more or less cut off from the general somatic fluid. This same method of distribution of nutritive material must also obtain between the endoderm and the ectoderm, the interchange being facilitated by the numerous pores which may be seen to penetrate the supporting lamella when horizontal sections of that structure are examined with the highest powers. But the stored nutritive material of the foot can only be rendered available for the body generally through the agency of the somatic fluid, and to a certain extent histological facts support this conclusion.

If we attempt to follow the further fate of the nutritive spheres we find that two things may happen to them. They may either undergo a gradual disintegration, their substance becoming at the same time studded with pigment grains which, after the nutritive sphere as such has ceased to exist, remain as a little heap of dark granules, bound together by a scanty amount of unstaining substance (fig. 25), while, as the dis-



integration of the sphere approaches completion, the vacuole in which it lay becomes obliterated; or they may be rapidly and entirely dissolved and discharged from the cells, the vacuoles meanwhile persisting and leaving the striking honey-combed appearance described above (fig. 22). Whatever may happen to the constituents of the sphere in the first case, which agrees with the fate of the nutritive spheres of *Hydra* as described by Kleinenberg and Greenwood, we can only conclude that in the second case they have been dissolved and discharged into the enteric cavity. It is even possible that we may divide the endoderm-cells, other than the gland-cells, into two sets: (1) Those which are concerned in the elimination of waste matter from the nutritive spheres or from the somatic fluid directly. These are the apical cells of the villi and the vacuolate cells in their immediate neighbourhood. And (2) Those which discharge their stored material, leaving, so far as can be detected, no residue. These lie towards and between the bases of the villi in the blastostyles and middle regions of the body and foot.

This conclusion, which may be accepted as a provisional hypothesis until the processes taking place in the endoderm shall have been worked out more fully, is based upon two facts; namely, that the pigment, as was noted by Allmann, is located only in the cells near the free ends of the villi, and that the bubbly cells in all their various conditions of incomplete or complete discharge always lie between or towards the bases of the villi.

Another point of evidence in favour of the view that the somatic fluid conveys stored nutriment from one part of the body to another is derived from a study of the histology of the spadix of the gonophores.

Structure of the Spadix of a Gonophore.—This, in the completely formed female gonophore, is composed of a considerable number of tongue-shaped villi, which have their apices turned towards the axis of the spadix, and project a considerable distance downwards towards the centre of the blastostyle. The cells between their bases, and therefore



forming the apex of the spadix, are long, narrow, and columnar in character; and their protoplasm is filled with numerous small vacuoles, the contents of which become precipitated by preserving reagents, thus conferring a characteristically turbid character on the entire cell. That end of the cell which abuts on the basement membrane separating the endoderm of the spadix from the developing generative elements is excavated to form a vacuole. If we examine the cells which form the villi we find that they have fundamentally the same structure, except that the basal vacuole is now so much elongated that we may almost speak of that portion of the cell as being canalised (fig. 23). In other words, the whole spadix is a specialised structure for the absorption of nutriment from the somatic fluid of the blastostyle, which nutriment is doubtless largely derived from the stored material of the vacuolate cells, through the help of the somatic fluid.

The absorbed nutriment, probably after it has undergone important changes at the hands of the cells of the spadix, is discharged into the vacuoles at their base which abut on the supporting lamella, whence it passes to supply the remarkably abundant fluid present in the entocodon of the female gonophore.

These different points—(1) the general distribution of the nutritive spheres, (2) the method of the discharge of those bodies, and (3) the fact that the gonophore possesses an organ, the spadix, the histological characters of which lead us to suppose that it is designed to absorb nutriment from the somatic fluid (which, especially in the autumn, when *Myriothela* appears to exist largely at the expense of its stored material, and probably throughout the year, must be largely recruited from the reserve material of the vacuolate endoderm-cells)—although when taken singly they are of slight value, yet when considered together and as mutually supporting one another they justify the statement that the metabolic activities of the different parts of the endoderm are brought into relation with one another through the agency of the somatic fluid.

If no such link existed, and if, therefore, the animal were unable to direct its entire resources towards the accomplishment of any metabolic act, we should expect to find evidence of the fact in the more marked exhaustion of the endoderm during starvation in the immediate neighbourhood of a gonophore as compared with the other parts of the blastostyle or of the body generally. But such evidence appears to be wanting.

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## EXPLANATION OF PLATES XXXVI & XXXVII,

Illustrating Mr. Hardy's memoir "On some Points in the Histology and Development of *Myriothele phrygia*."

FIG. 1.—Section through the ectoderm of the distal portion of a blastostyle. [Animal killed in May.]

FIG. 2.—Section of the ectoderm of the gonophore-bearing region. [Animal killed with osmic acid in May.]  $\frac{1}{16}$ th ob.

FIG. 3.—Teased preparation from the same specimen. The primitive germ-cells have dropped out.  $\frac{1}{16}$ th ob.

FIG. 4.—Section through the generative region of an exhausted animal killed in autumn.

FIGS. 5, 5a, 5b.—Ectoderm elements isolated by teasing. Osmic acid. In 5 and 5a are represented parts of the nerve network.

FIG. 6.—Isolated primitive germ-cells. Osmic acid.  $\frac{1}{18}$ th ob.

FIG. 7.—Section showing the process of absorption of the supporting lamella.

FIG. 8.—First stage in the formation of a gonophore. Ectoderm thickened and containing a cluster of primitive germ-cells in its lowest part.

FIG. 9.—The second stage in the formation of a gonophore.

FIG. 10.—The third or blastema stage.

FIG. 11.—A completely formed young gonophore.

FIG. 12.—Piece of the cuticle which covers the ectoderm stripped off.  $\frac{1}{16}$ th ob.

FIG. 13.—A *Myriothela* bearing buds in various stages.

FIG. 14.—Section through ciliated cell zone of the endoderm.

FIG. 15.—Goblet-cell. Osmic vapour.

FIG. 16.—Villus of goblet-cell zone.

FIG. 17.—Two apical cells.

FIG. 18.—Loaded gland-cell and empty vacuolate cell.

FIG. 18*a*.—Discharged gland-cell.

FIG. 18*b*.—Intermediate condition of gland-cells.

FIG. 19.—Group of small cells from apex of villus.

FIG. 20.—Oral end of *Myriothela* with lip retracted.

FIG. 21.—Villus from middle region of the body, with gland-cells just commencing to discharge.

FIG. 22.—Group of cells from the endoderm of a blastostyle. Osmic acid.

FIG. 23.—Villus from the spadix of a gonophore, showing canal-like vacuoles in the lower portion of the cells. At *a* and *a'* these are cut across.

FIG. 24.—Two cells from the endoderm of a blastostyle. Osmic acid.

FIG. 25.—Nutritive spheres containing pigment granules. Osmic acid.  
 $\frac{1}{18}$ th ob.

FIG. 26.—Body from the endoderm of a tentacle.

FIG. 27.—Endoderm-cell containing the large type of nutritive sphere.

FIG. 28.—Large nutritive sphere.





On the Structure of an Earthworm allied to  
Nemertodrilus, Mich., with Observations  
on the Post-embryonic Development of  
Certain Organs.

By

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With Plates XXXVIII and XXXIX.

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**I. Introductory.**

THE investigations of Rosa (5),<sup>1</sup> Michaelsen (3, 4), and more recently of myself (1), have shown that the earthworm fauna of tropical Africa is characterised by an extraordinary abundance and diversity of types of the family Eudrilidæ. This family had been known for a long time by the genus *Eudrilus* alone, which seemed to occupy—principally on account of the structure of the reproductive system—a somewhat isolated position among the Oligochæta.

<sup>1</sup> The numbers in brackets refer to the list of papers on p. 541.

Eudrilus itself has been only lately received from the African continent; but no less than eight new genera of this family have been within the last year recorded from thence. I have myself on two separate occasions received worms from Lagos, which proved to be the types of three new genera; this shows the great abundance of forms which must remain to be discovered.

In a recent number of this Journal (1) I described two genera—*Hyperiodrilus* and *Heliiodrilus*—which I obtained from the Royal Gardens at Kew; they were found in earth which had come from Lagos. In all probability the genus *Siphonogaster*, of which I have given a brief notice in the last published number of the 'Proceedings of the Zoological Society,' will prove to be a *Eudrilid*; this worm was discovered by Mr. Alvan Millson, Assistant Colonial Secretary at Lagos, who has contributed to the 'Kew Bulletin' of October, 1890, a very interesting account of its habits. The same gentleman was so good as to bring over with him a large box full of living earthworms from Lagos, which included some specimens of the "Yoruba worm;" unfortunately these latter disappeared during the voyage, but the other species survived; it proves to be the type of a perfectly distinct and new genus, which presents some remarkable peculiarities of structure. In the box were also some examples of a small worm, which appears to be an *Allolobophora*, and some very young examples of the new *Eudrilid*. The opportunity thus afforded me of contributing something towards the development of the reproductive system and nephridia of *Libyodrilus violaceus* causes me to feel less regret that it was impossible to keep the species alive in order that they might breed.

I am greatly indebted to Mr. Alvan Millson for his kindness.

## II. List of Memoirs consulted.

- (1) BEDDARD, F. E.—“On the Structure of Two New Genera of Eudrilidæ, with Remarks on Nemertodrilus, Mich.,” ‘Quart. Journ. Micr. Sci.,’ March, 1891.
- (2) BEDDARD, F. E.—“On the Occurrence of Numerous Nephridia in the same Segment in Certain Earthworms, and on the Relationship between the Excretory System in the Annelides and in the Platyhelminths,” ‘Quart. Journ. Micr. Sci.,’ vol. xxviii, N. S., p. 397.
- (3) MICHAELSEN, W.—“Beschreibung der von Herrn Dr. Franz Stuhlmann im Mündungsgebiet des Sambesi gesammelten Terricolen,” ‘Jahrb. Hamb. Wiss. Anat.,’ Bd. vii.
- (4) MICHAELSEN, W.—“Oligochaeten des Naturhistorischen Museums in Hamburg,” iv; *ibid.*, Bd. viii.
- (5) ROSA, D.—“Lombrichi dello Scioa,” ‘Ann. Mus. Civ. Genova,’ 1888.
- (6) HORST, R.—“Note on a New Earthworm,” ‘Zool. Anz.,’ 1891.
- (7) HORST, R.—“Sur quelques Lombriciens exotiques appartenant au genre Eudrilus,” ‘Mém. Soc. Zool.,’ 1890, p. 223.
- (8) CERFONTAINE, P.—“Recherches sur le Système Cutané et sur le Système Musculaire du Lombric Terrestre (*Lumbricus agricola*, Hoffmeister),” ‘Mém. cour. et Mém. sav. étr. Acad. Roy. Belg.,’ 1890. (This paper is reprinted in ‘Arch. de Biol.’)
- (9) SPENCER, W. B.—“The Anatomy of *Megascolides australis* (the Giant Earthworm of Gippsland),” ‘Trans. Roy. Soc. Victoria,’ vol. i, part 1.
- (10) PERRIER, E.—“Mémoires pour servir à l’histoire des Lombriciens terrestres,” ‘Nouv. Arch. Mus.,’ t. viii.
- (11) BENHAM, W. B.—“Atrium or Prostate?” ‘Zool. Anz.,’ 1890.
- (12) D’UDEKEM, J.—“Histoire Naturelle du Tubifex des Ruisseaux,” ‘Mém. cour. et Mém. des sav. étr.,’ 1853.
- (13) HORST, R.—“Descriptions of Earthworms,” I, ‘Notes Leyden Mus.,’ vol. ix, p. 97.
- (14) EISIG, H.—“Die Capitelliden,” in ‘Fauna and Flora des Golfes von Neapel.’
- (15) BEDDARD, F. E.—“Contributions to the Anatomy of Earthworms,” No. I, ‘Proc. Zool. Soc.,’ 1887.

### III. External Characters.

The worms were brought over in a large wooden box nearly filled with a sandy loam; they preferred the bottom of the box, and were in nearly every case found in close contact with the wood; this may be perhaps owing to the greater warmth. The worms were very inactive in their movements; but this is possibly not so much a characteristic of the species as due to the coldness of the weather. Nevertheless I have not found that tropical *Perichæta*, which are the most agile of earthworms, are in any way influenced by such causes.

The colour of the species is a dull purplish brown with a distinct pinkish tinge; the clitellum is only to be distinguished by its darker colour. The colouring substance was largely dissolved out by alcohol staining the fluid of a greenish yellow. There remained, however, after treatment with alcohol, a dark coloration on the dorsal surface.

The worms, when placed in spirit, protruded the buccal cavity, which appeared of a bright red colour; they do not protrude it in locomotion, as *Perichæta* does.

**Prostomium.**—The prostomium is short, and not continued by a groove on to the buccal segment.

**Setæ.**—The setæ are strictly paired, and lie upon the ventral surface of the animal. There is nothing unusual in their form. The ventral setæ of Segment 17 are absent, as shown in the illustration (fig. 2). The lateral and ventral setæ of each side of the body are connected by a muscular slip shown in fig. 19, *M*.

Dorsal pores appear to be, as in other Eudrilids, entirely absent.

Nephridiopores, usually conspicuous enough in this family, could not be detected in the present species (see, however, p. 556) even with a lens.

The clitellum is continuously developed all round the body: in most specimens it occupied only two segments, viz Nos. 15 and 16; in some, however, it extended over the 14th



also. The extent of the clitellum is more limited than in other Eudrilids, where four to six segments appear to be the rule.

**Spermathecal Pore.**—This is situated in the middle ventral line (see fig. 2, *sp*) of Segment 13; it lies between the ventral pairs of setæ; the aperture itself is small, but its position is rendered somewhat conspicuous by being placed upon the summit of a slight swelling of a yellowish colour.

**Oviducal Pores.**—These pores occupy what is, at present, an unique position among earthworms. With the exception of certain species of *Moniligaster*, and with the possible exception of *Eisenia* (= *Tetragonurus* of Eisen), the oviducts of earthworms appear to open invariably upon the 14th segment. In the present species they are situated on Segment 15. I took particular pains to assure myself that this was really the case; in half a dozen or so of individuals which were marked by having these orifices very conspicuous they were certainly situated on that segment. The oviducal pores in this worm are not difficult to see; indeed, these apertures are for the most part particularly plain in the Eudrilidæ. This is due to the fact that the end of the oviduct is often slightly protruded, and forms a round whitish elevation contrasting in colour with the surrounding dark integument. I mention these facts more particularly for the purpose of showing that I am not likely to have fallen into an error in assigning this abnormal position to the oviduct pore. The pore in *Libyodrilus* has the same appearance as I have figured and described in *Heliodrilus* (1); that is to say, it is a whitish knob upon the segment: it lies to the outside of and a little in front of the lateral pair of setæ, but well behind the groove which separates its segment (the 15th) from the 14th. These are the only apertures belonging to the genital system which are paired.

The male genital pore is unpaired and median; it lies on the border line of Segments 17, 18. The aperture lies upon the summit of a conspicuous elevation of the integument; the

two penial setæ may be often seen protruding from the orifice.

I found no genital papillæ of any kind.

#### IV. Anatomy and Histology.

##### § Body-wall.

**Epidermis.**—The chief point to be noted about the epidermis is the absence of the peculiar sense organs so generally found among the Eudrilidæ. I have described them in *Eudrilus*, *Hyperiodrilus*, and *Heliodrilus*. Dr. Horst has recently described them in an African species of *Eudrilus*, and was the first to suggest that they were possibly sense organs; he compares them to the Pacinian bodies of Vertebrata. I had originally considered these bodies as representing rudimentary setæ, and as corresponding to those epidermal structures in *Urochæta* which Perrier first described, and considered as rudimentary setæ; but a more detailed examination of their structure led me to express in my paper upon *Hyperiodrilus* and *Heliodrilus* an opinion that they were of a sensory nature, and to make the identical comparison that Dr. Horst made, previously in point of time of publication. Rosa has found these structures in *Teleudrilus*; but they do not exist in *Nemertodrilus* or in *Libyodrilus*. The absence of these epidermal sense organs unites these two genera, which have other points in common.

The cells of the epidermis are of the usual kind; the large glandular cells appear to be perhaps unusually abundant. I have not made a special study of the histology of the epidermis, which has been recently so elaborately worked out and so beautifully illustrated in *Lumbricus* by M. Cerfontaine (8).

**Muscular Layers.**—The usual circular and longitudinal muscles are present; the relative thickness of the two coats may be judged from figs. 4 and 13. In both layers the fibres

are embedded in a gelatinous-looking matrix, which is more abundant in proportion to the muscular tissue in the case of the longitudinal coat. As to the fibres themselves, I find that, as M. Cerfontaine has described in *Lumbricus* (8), both those of the longitudinal and circular coat present a radiately striate appearance, the striæ converging towards a central clear space. I have not taken any special means to investigate thoroughly the histological structure of the muscles, I only describe what I have seen in transverse and longitudinal sections of the entire worm; but I have little doubt from what I have been able to see that the results of M. Cerfontaine's investigations into *Lumbricus* will hold for *Libyodrilus* also. The matrix in which the fibres are embedded can hardly be termed granular in *Libyodrilus*; it has a gelatinous homogeneous appearance, and is but slightly affected by borax carmine. It forms a specially thick layer (fig. 14) between the end of the longitudinal muscular fibres and the peritoneum. The fibres—both those of the circular and the longitudinal coat—tend to be grouped into definite areas, which are only separated from each other by the thickness of the matrix lying between them. I quite agree with M. Cerfontaine that there is nothing comparable to the septa described by Claparède. No doubt such an appearance might be produced by great shrinking, caused by preservation in strong alcohol without previously fixing the tissues.

The arrangement of the fibres of both muscular coats is regular, but the regularity is not so great as in certain species of *Lumbricus* and *Allolobophora*; each "compartment" is made up of fibres, which are three, four, or five deep—even more sometimes.

It is an interesting fact that in the young worm the fibres are disposed much more nearly in the bipinnate fashion originally made known by the descriptions of Claparède.

### § Cœlom and Intersegmental Septa.

The first septum separates Segments 4—5; in front of this the pharynx is attached to the parietes by numerous muscu-



lar bands which occupy a good deal of the coelomic space: it is probable that the reduction of the nephridia in this region of the body is brought about by the development of this extensive meshwork of muscles. The septa which lie behind the first are thickened, and formed often of distinct layers of fibres. The last of the thick septa separates Segments 11—12; the next septum, however (see fig. 1), although not so thick as those which precede it, is thicker than those which follow.

A noteworthy point is the non-coincidence of the insertion of the septa with the intersegmental furrows. This frequently occurs in earthworms, but according to Rosa it is possible to trace the fibres belonging to the septum from their apparent attachment to the middle of the segment to their real insertion into the intersegmental furrow. I could not trace any such arrangement in *Libyodrilus*. The first septum which has an abnormal insertion is 12—13; this and the following six or seven are attached near to the middle of the segment just in front of the setæ. It is only dorsally and laterally that the septa are so attached; ventrally their insertion is quite regular. I have mentioned on page 571 the effect produced upon the position of the oviducal pore by the shifting of the intersegmental septum 14—15.

The principal point of interest in connection with the coelom is the separation of spaces which surround some of the organs. In my description of the vascular system I have referred to the perihæmal space which encloses the subœsophageal blood-vessels; besides this space, the areas surrounding both of the lateral pair of setæ are enclosed by a membrane shutting them off from the general body-cavity of their segments. A series of transverse sections, such as the one illustrated in fig. 19, shows that this membrane is perfectly continuous everywhere, forming a distinct chamber, which is prolonged round the seta sac and nearly reaches the circular muscular coat. In the *Capitellidæ* Eisig has described somewhat similar lateral chambers, into the floors of which the setæ are inserted; but I am not aware that anything of the kind has until now been



described in the *Oligochæta*. As will be seen from fig. 19, the muscle connecting the two pairs of setæ lies within the chamber. The membrane which forms the walls of the chamber is nearly homogeneous in appearance, with a covering and lining of epithelium only recognisable by the nuclei.

### § Alimentary Canal.

The alimentary canal of *Libyodrilus* presents certain important differences from that of other *Eudrilidæ*. There is in the first place no trace that I could discover of œsophageal glands, or of those impaired ventral pouches termed "chylus-taschen" by Michaelsen, which occur in so many *Eudrilids*.

The position of the three gizzards is another point of interest. In *Heliodrilus* and *Hyperiodrilus* there are a larger number of gizzards, which are situated as in *Lumbricus* at the junction of the œsophagus and intestine; the three gizzards of *Libyodrilus* occupy a similar position.

In his memoir upon the classification of earthworms Perrier remarked upon the occurrence in all the *Anticlitellians* of the gizzard behind the organs of generation and the contractile hearts of the vascular system; from this group he distinguished both the *Post-* and *Intra-clitellian* worms by the fact that the gizzard was placed in front of the essential organs of reproduction.

This distinction held good until my own discovery of the position of the gizzards in the two *Eudrilids* above referred to. In some forms—for instance, a *Teleudrilus*—the gizzard has the forward position which characterises the majority of worms belonging to Perrier's two groups of *Intra-clitellians* and *Postclitellians*; but I am disposed to think that the arrangement of these organs found in *Heliodrilus*, *Hyperiodrilus*, and *Libyodrilus* will prove to be more characteristic of the group. There are no particular reasons that I can see for placing the *Eudrilidæ* in the neighbourhood of the *Lumbricidæ*, and thus one of the characters of the latter group can no longer be regarded as distinctive. Dr. Horst

has already shown in his brief account (6) of an evidently very interesting form, *Glyphidrilus Weberi*, that the position of the clitellum hitherto found only among the Lumbricidæ is also to be found in a worm clearly referable to Perrier's *Intraclitellians*. The varying position of the gizzard is less remarkable now that there is some probability of its epithelium being always derived from the hypoblast; if the older view that the gizzard belonged to the stomodæal invagination had been confirmed by more recent researches, it would have been necessary to regard the calciferous pouches as being epiblastic when in front of the gizzard and hypoblastic when lying behind it. The relations of the same diverticula show that when the gizzard is followed immediately by the "large intestine" it does not mean that there has been a suppression of the small intestine immediately following the gizzard. The absence of any relation between the position of the gizzard (or gizzards) and the position of other organs seems to show clearly that this modification of the walls of the anterior section of the gut may take place anywhere. There is no need to assume that the gizzard of one earthworm is the exact homologue of that of another.

The pharynx occupies the first five segments.

The œsophagus is of great length, and divided into two distinct sections, neither of which, as already mentioned, is furnished with glandular diverticula. The first section of the tube is of narrower calibre, and extends as far as the end of Segment 19. After this the tube becomes suddenly dilated and forms a kind of crop, which immediately precedes the gizzards. The "crop" occupies Segments 20—22 inclusive.

As to histological structure, the œsophagus does not differ from that of other earthworms; its walls are exceedingly vascular, and the epithelium is in many parts rather thin. Even in the 12th segment there are a few chloragogen cells upon the œsophagus. These become largely developed in Segment 15, where the œsophagus is as closely enveloped by them as is the intestine; the transition, however, appears to be gradual. The latter part of the œsophagus from about Segment

14 onwards is ciliated ; its epithelium is thrown into regular longitudinal folds ; the cells are tall and columnar, and the muscular coat is of an appreciable thickness.

The œsophagus is extremely vascular ; in longitudinal and transverse sections its walls have from this cause a moniliform appearance (fig. 16). So far as I could make out, the plexus of blood-vessels which lies immediately below the epidermis was nowhere a sinus, although the individual vessels were so close together as to give this appearance ; still a more careful examination showed the longitudinally running connecting branches between the much wider circular vessels.

The three gizzards are in Segments 23, 24, and 25—one gizzard to each segment ; they are not all three directly continuous, but are separated by a certain amount of soft-walled intestine. The soft-walled portion occupies the anterior part of each gizzard segment ; posteriorly, the gizzard comes into contact with the septum.

The width of the crop gives the gizzards the appearance of being strung, as it were, upon the intestine ; but that the crop belongs to the œsophagus and not to the intestine is shown by the fact that it has no typhlosole.

The intestine is furnished with a typhlosole which is of a somewhat unusual form. From its very commencement to as far back as Segment 37, three folds can be seen on a dissection—one median, and one on each side. The median fold corresponds, of course, to the dorsal vessel, and is of about thrice the vertical diameter of each of the two lateral folds. All three folds were quite conspicuous in dissections from their red colour. After the 37th segment the two lateral folds disappeared, but the median typhlosole continued on to near the end of the body.

In dissected worms, both fresh and after preservation, the intestine was of a rich mahogany brown colour, quite different from the colour of our British *Lumbrici* ; *Nemertodrilus* and some of the other *Eudrilids* agree with the present species in this particular, which is due to the abundance and darkness of the granules in the chloragogen cells.



### § Circulatory System and Perihæmal Spaces.

The principal account of the circulatory system in this family of Oligochaeta—and that is very incomplete—is contained in a paper upon *Eudrilus* by myself (15). The blood-vessels of *Libyodrilus* are very similar, but I did not find a sub-nervian trunk. Very frequently in transverse sections a blood-vessel could be seen lying beneath the peritoneal membrane, and vertically beneath the nerve-cord; but by following out the vessel through a few sections it was invariably found to be merely a part of the integumental blood plexus running for a short distance in a longitudinal direction. The dorsal vessel is united with the ventral by a series of paired “hearts.” These increase in size posteriorly. The dorsal vessel is ensheathed by a layer of “chloragogen” cells, beneath which is a thin layer of very delicate muscular fibrils; the apertures of the hearts into the dorsal vessel are guarded by valves consisting of a mass of granular columnar cells with small nuclei; there are similar valves where the hearts debouch into the ventral vessel. The supra-intestinal trunk is recognisable only in the œsophageal region of the worm; it is very easily seen in dissections, and its calibre is quite equal to that of the dorsal vessel. The supra-intestinal differs from the dorsal blood-vessel in having only a very thin peritoneal layer; there are no large chloragogen cells like the pear-shaped cells which cover the dorsal vessel, only a covering of much flattened cells: the supra-intestinal trunk is united with the dorsal by a vertical sheet of mesentery. The supra-intestinal vessel is directly concerned with the blood supply of the œsophagus. As already mentioned, the œsophageal walls contain an extremely rich meshwork of blood capillaries; in specimens that happen to have been killed with this part of the vascular system naturally injected, the walls are rendered turgid by the contained capillaries, which have almost the appearance of forming a continuous sinus; here and there the supra-intestinal vessel is connected with this network by a short tube.

The presence of infra-œsophageal vessels has been



described by myself in *Eudrilus*; as they occur in *Libyodrilus* they will probably prove to be characteristic of the family. Similar vessels appear to occur in other earthworms, but their distribution has not been much studied. In *Libyodrilus* as in *Eudrilus* they are paired trunks, but they do not unite together at intervals as in the latter genus. These vessels are, like the supra-œsophageal (supra-intestinal) trunk, concerned with the blood supply of the œsophagus; they are probably efferent trunks, taking the blood away from the œsophagus, which has reached it by way of the supra-œsophageal vessel.

Two mesenteries are connected with these vessels and with the ventral œsophageal wall; these enclose a space which appears to be completely shut off from the cœlom of the segments. The arrangement of these mesenteries differs in different parts of each segment. Towards the middle of the segment, as is shown in fig. 17, the two mesenteries originate from the lateral regions of the œsophagus (the histological details of the œsophagus are not shown in the figure); they are thrown into folds by the contraction caused by the preserving fluid. These mesenteries unite considerably above the ventral blood-vessel, and thus enclose a space which is roughly crescentic in outline but of considerable vertical depth. The membrane consists of a faintly staining nearly homogeneous core, covered on both sides by peritoneal cells, of which the nuclei were alone visible; here and there small groups of longitudinally running bands of muscular fibres, not shown in the figure cited, are embedded in this homogeneous layer. The figure, however, does show two longitudinally running bands of muscle (*m*); these are special muscles attaching the ventral surface of the œsophagus either to the septa or to the ventral parietes. Above these, i. e. nearer to the œsophageal wall, are the paired sub-œsophageal trunks (*bl. ves.*); in the section represented in the figure the two blood-vessels are seen to be attached to the mesenteries, but in other sections they lie freely within the mesenteries, and further back they may come to lie outside. These facts appear to show that

we are dealing here with a perihæmal cœlomic space not found in connection with supporting mesenteries of the blood-vessels; further forward in the segment the two lateral membranes come to be attached to the intersegmental septum where this forms the floor of the perihæmal chamber. The supra-œsophageal vessel is for the most part not enveloped in a perihæmal space, but it is connected in parts by the membranes with the walls of the œsophagus; these gradually shift their areas of attachment to the œsophagus until they are nearly continuous with the two membranes which enclose the sub-œsophageal vessels.

The formation of perihæmal spaces is an interesting, but not a perfectly new fact in the anatomy of Oligochæta.

I believe that I was the first to call attention to the fact that, in an earthworm (*Deinodrilus*) belonging to quite a different family, the dorsal vessel is for the greater part of its course enclosed in a "pericardium." An almost identical structure has been figured by Professor Spencer in *Megascolides australis*. It is noteworthy that in both these cases the "pericardium" contains abundant perivisceral corpuscles which stain deeply, and appear to be merely the younger forms among the corpuscles of the perivisceral fluid. I suggested the possibility of these corpuscles being principally formed in that space.

Similar corpuscles occur in the space which surrounds the supra-œsophageal vessel in *Hyperiodrilus* and *Heliodrilus*, and I have found them in *Libyodrilus*. I did not, however, observe any proliferation of these cells from the lining membrane of the perihæmal space in *Libyodrilus*; there were simply masses of these cells (fig. 17, *corp.*) lying here and there within the space.

Fig. 21 represents the course of the sub-œsophageal vessels in Segments 9—11.

They generally lie at some distance from the œsophageal wall, and are connected at frequent intervals by branches with the peri-œsophageal plexus. Towards the anterior part of Segment 9 the two trunks are connected by a branch which

was prolonged backwards and downwards to the neighbourhood of the ventral vessel; this branch (*a*) does not, however, open into that vessel, but runs into one of the muscular bands which unite the œsophagus with the parietes. Farther back still each vessel gives off a branch which supplies the inter-segmental septum; here its capillaries no doubt unite with those of branches of the ventral blood-vessel. In the 10th segment there was no connection between the two sub-œsophageal trunks; each of them gives off a branch (*a*) which supplies the muscle (*m.*) running from the œsophagus to the parietes.

These branches pass along the walls of the perihæmal space and reach the interior of the muscle, along which they pass to its point of insertion; these two branches, of course, correspond to the single cutaneous branch which I have described in Segment 9. At the septum dividing this segment from the 11th a branch is given off from each vessel, which supplies not only the septum but also the sperm-sac. In the next segment the branches of the sub-œsophageal vessels are the same, and therefore need no description. I imagine that these sub-œsophageal vessels represent, wholly or in part, the lateral trunks<sup>1</sup> of other earthworms, which appear at least frequently, if not always, to take their origin from the peri-œsophageal blood network.

### § Nephridia.

In dissecting the worm the nephridia were quite obvious in most of the segments of the body; and where visible, presented a paired arrangement which characterises all the Eudrilidæ. In the posterior region of the body the nephridia were particularly conspicuous, both in fresh and alcoholic specimens, by reason of their opaque white coloration. In the anterior segments of the body the nephridia were largely adherent to the posterior septum of their segments, and did not show the white opaque appearance of the posterior nephridia. We have evidently here another instance of what

<sup>1</sup> "Intestino-tegumentary."

is very common among the terrestrial Oligochæta, viz. a separation of the nephridia into two series, an anterior and a posterior; this is seen, among other forms, in *Pontodrilus* and in *Microchæta*. In the segments occupied by the spermatheca, viz. in Segments 13—17,<sup>1</sup> no trace of nephridia could be detected in a dissection. I am disposed to refer this partial obliteration of the nephridia in this region of the body (we shall see immediately that they have not absolutely disappeared) to the enormous development of the spermathecal sac, which occupies most of the available space; the case is, in fact, analogous to that of many aquatic forms in which the nephridia disappear with the appearance of the genital organs.

Each nephridium (fig. 11, *f.*) has a funnel lying, as usual, in the segment anterior to that which bears the external pore. The nephridia of the posterior segments are enveloped by a mass of peritoneal cells; such at least appears to be the nature of the very peculiar tissue illustrated in fig. 10, *c.* I may remark here that both *Heliodrilus* and *Hyperiodrilus* have a similar investment to the nephridia. A covering of this kind which is simply an exaggeration of the ordinary peritoneal layer is very characteristic of the aquatic Oligochæta, but it has before now been recorded in earthworms; it was first described in an earthworm by Perrier in *Pontodrilus*.

In sections of *Libyodrilus* which have been stained with borax carmine this mass of cells is very deeply coloured; the staining fluid has, however, chiefly affected not the protoplasm of the cells, but innumerable spherical particles with which they are crowded. Among these deeply-stained spherules are masses of others, rather larger, which are not stained at all (cf. fig. 6, *c'*). The cells were not, however, always found to be loaded with the products of their activity; occasionally the nephridial tube is embedded in a mass of deeply-staining cells, of which the nuclei were very conspicuous; the out-

<sup>1</sup> There was some variation, probably due to different stages of maturity, in the number of segments from which nephridia were apparently absent.



lines of the cells were not in every case so clear. Comparatively few of the cells contained agglomerations of secreted spherules. The worm which furnished the material for the section described here was immature; but it must not be inferred from this that the activity of the glandular cells surrounding the nephridia does not commence until the animal is fully mature; in the youngest individual which I have been able to study the cells in question were crowded with spherules. Their activity is evidently intermittent.

In fig. 11 are represented three successive nephridia from the post-clitellar region, belonging to as many segments. The nephridia themselves are opaque white bodies, broader towards the middle line of the body as there represented; on the upper surface it is quite easy to see, even without using a lens, a single loop which appears darker than the surrounding tissue on account of the thinness of its walls. This part of the nephridium will be referred to in connection with the minute structure of the glands. A narrow tube leading from the broad end of the nephridium and perforating the septum traced in the funnel, situated in the segment in front, is quite easy to make out; so is another tube which passes direct to the body-wall in front of the ventral pair of setæ; this tube is the external duct of the nephridium. Anyone who was contented, as were some of the earlier investigators, with dissection only, would be satisfied that in such a drawing as fig. 11 the nephridium was sufficiently displayed. The nephridium itself is shown, the funnel and the duct leading to the exterior. But there is apparently no external orifice corresponding to the place where the duct appears to perforate the body-wall on its way to the exterior.

In examining the body of the worm with a lens I did not succeed, as already mentioned, in discovering the nephridiopores, which are generally quite easily visible, even without a lens.

This failure to find the nephridiopores was at once accounted for when I examined a fragment of the cuticle stripped off and mounted in a drop of water.

Besides the minute orifices of the unicellular glands of the epidermis (fig. 3), which here, as in other species, show a cross-like form illustrated in many memoirs upon the anatomy of earthworms, there were other larger orifices of a circular or perhaps slightly elliptical form. In the figure cited the number of these apertures relatively to those of the unicellular glands is shown; they are extremely numerous in each segment, and have no particular regularity of arrangement.

These apertures obviously suggest the external pores of the nephridia, which are so numerous in *Perichæta* and other forms with diffuse nephridia. In *Perichæta* the apertures in question are of moderate size, though considerably smaller than the apertures through which the setæ protrude. They are continuous with a little cylinder of chitinous membrane, which is, of course, the lining of the nephridial tube.

In *Libyodrilus* the apertures that I have referred to were smaller than those of *Perichæta*, and I could not detect the involution of the chitinous membrane. Otherwise I should have thought myself justified in stating, if necessary without any further investigation, that *Libyodrilus* belonged to that group characterised by a diffuse, irregular nephridial system.

This suggestion, however, appears to be somewhat at variance with the statement that *Libyodrilus*, like other *Eudrilids*, possesses paired nephridia, one pair to each segment. I have found, however, by a series of sections, that the body-wall is permeated by a system of branching and anastomosing canals, opening on to the exterior by numerous apertures, and connected on the other hand with the paired nephridia. I took these canals at first for blood-vessels, to which they bear not a little resemblance; indeed, I am still unable to find any very marked histological difference between these tubes and the blood-vessels. That they belonged to a different system was shown by the fact that they were frequently found to be accompanied by blood-vessels. It is hardly perhaps necessary to say that there was no connection between these tubes and the blood capillaries; the former never contained blood-clots, while it was impossible to follow out a

blood-vessel through more than two or three consecutive sections without finding coagulated blood in its interior; and it is not difficult in well-preserved earthworms to detect the blood-clots, nor is it easy to confuse them with any other structures as a general rule.

The tubes that I have referred to consist of larger trunks with a definite arrangement, and smaller vessels which form a plexus; the longitudinal and circular muscular coats were permeated by them. It is, perhaps, necessary to explain here that the tubes in question have nothing to do with the "Lymphspalträume" which Dr. Kükenthal has figured and described in certain Oligochæta, particularly in *Tubifex*; I have met with spaces similar to those, and crowded with lymph-corpuscles, in various genera of earthworms. I found them to be particularly abundant in the two genera *Heliodrilus* and *Hyperiodrilus* described in a recent number of this Journal (1). They consisted in those genera, as Dr. Kükenthal has pointed out for other forms, of spaces, with no special walls, between the muscular fibres. I quite agree with Dr. Kükenthal in regarding these as the first beginning of the lymphatic system of the Vertebrata, in which group the finest branches of the lymphatic system are without intrinsic walls. The tubes which I describe here have definite though thin walls, darkly stained by borax carmine; the substance of which they are composed is granular in appearance, and there are evident nuclei attached here and there to the outside of the walls. The principal trunks run in a longitudinal and a transverse direction; there are four main longitudinal trunks, which run on a level with the four pairs of setæ; they are continuous from segment to segment.

These tubes are of considerable calibre, almost equal in diameter to one of the setæ, or even greater; it is not particularly useful to give accurate measurements of them, for the reason that they varied considerably in size from place to place, contracting here and expanding there. Their walls are thin, but quite as thick as those of blood-vessels of about the same size.



The longitudinal muscular coat consists, like that of other earthworms, of fibres embedded in a matrix of connective tissue. As I have already mentioned (*supra*, p. 545), this connective tissue forms a thickish layer below the level where the muscular fibres end; it is in this layer that the longitudinal vessels run. At the implantation of the couples of setæ the longitudinal vessels come to lie within the arch formed by the two setæ—which converge at their deep ends, and diverge superficially. Just behind the setæ the longitudinal vessel gives off a wide branch, which gradually gets nearer and nearer to the thin peritoneal epithelium. Ultimately (fig. 7) it comes to lie within the body-cavity, surrounded at first by a prolongation of the almost hyaline connective tissue of the longitudinal muscular layer. This covering finally disappears, and the tube comes to an end; in a few cases I have traced it into connection with a very rudimentary coil of nephridial tubules. I have already mentioned that in the clitellar segments the nephridia are not visible with a lens. The tube lying within the cœlom was always accompanied by a blood-vessel of about the same diameter, and I have observed the two to be suspended by a common mesentery. The longitudinal vessels are also provided with numerous branches, which pass among the longitudinal muscles; some of these branches are smaller, some larger; a few of the larger ones pass obliquely upwards in the direction of the circular muscular layer; the tract which these branches traverse is free from muscular fibres; traced upwards, these branches are seen to open into a circular vessel, which runs right round the body; these circular vessels, which are repeated metamerically, lie just between the circular and longitudinal muscular coats, close to an important nerve, which also passes right round the body (see fig. 20). The circular vessels are of considerable width, and can be easily recognised in sections examined by quite low powers; their outline is somewhat crenated, which probably means that they can be expanded or retracted in accordance with the movements of the body. The fact that the vessel lies between the two muscular coats, instead of within the substance of one of



them, probably ensures less injury through pressure. These tubes, like the rest, appeared to be entirely empty, or, at any rate, to be filled with an absolutely clear fluid; they give off numerous branches on all sides; the commencement of the branches, which run along the segments from end to end—that is, in a longitudinal direction—could be seen in transverse sections as elliptical foramina in the walls of the tube (see fig. 14). The branches which pass downwards ramify among the longitudinal muscles, and even on the dorsal side of the body of the worm. Where there are no conspicuous longitudinally-running trunks the branches reach the thick layer of connective tissue which lies to the inside of the longitudinal muscles; arrived there, they pass in different directions, longitudinal as well as transverse. Besides the main transverse trunks running between the two muscular coats I observed vessels of less calibre, running, as shown in the diagram (fig. 16, *a*), just below the peritoneum, and apparently continuous right round the body.

The branches which pass upwards could be traced as far as the epidermis; they were constantly, like the other branches, accompanied by blood-capillaries. I could not detect the actual orifices, but at frequent intervals the non-glandular cells of the epidermis were closely crowded together. At these points I imagine lie the external orifices which are so evident upon the cuticle when it is stripped off and examined.

In no case were cilia observable along the course of the tube. It is clear that this system of tubes forming a plexus within the muscular coats or the body-walls is a new form of excretory organ, and unlike any that has been hitherto described in any Oligochæte.

A plexus of nephridial tubes, not interrupted by the septa, occurs in a good many forms; but it has been hitherto found to lie in the cœlom, or is at most partly retro-peritoneal; besides, in the genera (*Perichæta*, *Megascolides*, &c.) which have excretory organs of that pattern the tubes are to some extent ciliated; in those forms there are numerous external pores, and so the integument is perforated by numerous nephridial tubes. These may even branch on their way to the

exterior, as I have figured in *Acanthodrilus* (2, pl. xxx, fig. 1), and as Prof. Spencer has figured in *Megascolides australis* (9, pl. vi, fig. 21); but there is no branching and anastomosis combined, such as is described above in *Libyodrilus*; in *Perichæta*, &c., the network is confined to the cœlom, and is formed out of the proximal part of the nephridia; in *Libyodrilus* the network is in the thickness of the body-wall, and is formed out of the distal part of the nephridia. It has been already explained that the paired nephridia of the clitellar segments become rudimentary, though they do not entirely disappear; if the cœlomic part of the nephridia were to disappear—and they have all but done so in the clitellar region—the nephridial system would consist merely of a rich network of smaller and larger tubes in the thickness of the body-wall.

The figure (fig. 14) which illustrates the arrangement of this portion of the excretory system represents a combination of several sections. It should be stated, however, that it is not meant as a diagram; such an arrangement as is there depicted might well occur and be visible in the thickness of any one section. To thoroughly demonstrate the network in the longitudinal muscular layer probably needs some process of injection which I have not been able to apply. Had I been aware of this interesting network of fine tubes in the skin I should have certainly attempted some injection while I had living specimens at my disposal. As it is, I am inclined to think that I have not in my drawing done full justice to the complexity of the meshwork; the scheme (fig. 16), which is purely diagrammatical, expresses my idea as to this network. In fig. 14 two longitudinal ducts (*d*) are shown, and the connection of each with the circular vessel: the latter (*c*) is represented as cut at different levels; the thickness of the walls of the tubes ramifying among the longitudinal muscles is, perhaps, rather exaggerated. At *a* a branch of the longitudinal trunk *d* is seen to leave the muscular layer, and to project into the body-cavity, still surrounded by a connective-tissue sheath; this branch joins one of the paired nephridia.

Fig. 7 represents one of the longitudinal trunks seen in longitudinal section; it runs for the greater part of its course in the thick layer of gelatinous tissue which bounds the longitudinal muscles below; at the implantation of the setæ it passes up into the muscular layer itself. These vessels, as will be seen from the figure, have a somewhat undulating course; they are occasionally diverted by a large blood-vessel. I have not shown in this figure many branches passing from the longitudinal trunk, because these were not visible, except at *a*, in the section of which it is a drawing. At *N* the duct of a nephridium is seen to join the longitudinal vessel. In the anterior segments of the body the ducts of the nephridia pass down in close contact with the hinder septum of their segment to join the integumental network; in the posterior segments, as is shown in fig. 7, they are not in such close relation to the septum.

A connection of the nephridia with the pharynx occurs in this earthworm. The ventral wall of the pharynx, as in other *Oligochæta*, is formed of but little more than the lining epithelium; the dorsal wall, on the contrary, is extremely thick, owing to the mass of muscles which overlies the epithelium. Here and there the epithelium of the ventral pharyngeal wall gives off very short tubular diverticula which are connected with nephridial tubules; these same nephridial tubules can be traced in the other direction into the longitudinal vessels of the body-wall and so to the exterior (fig. 15).

An opening of nephridia into the stomodæal region of the alimentary tract in earthworms was first discovered by myself in *Acanthodrilus multiporus*; subsequently Spencer found numerous nephridial apertures into the pharynx in *Megascolides*.

In fig. 15, the opening of a tubule into the ventral side of the pharynx is shown; these apertures are not numerous, and appear to be regularly paired; the epithelium of the pharynx dips down, its cells gradually getting shorter; the nephridium, into which this short diverticulum opens, forms a coil within a



layer of connective tissue and muscles belonging to the pharyngeal wall.

The development of these nephridia, so far as I have been able to trace it (see on p. 574), seems to indicate that the network in the body-wall is a secondary arrangement. It is undoubtedly necessary to be very careful in arguing from ontogeny to phylogeny, but so far as the facts go they seem to point in this direction. In this case it will be hardly permissible to compare the network to that of flat-worms, particularly of Cestodes; in the Cestodes the nephridial network appears to exist not only in the mesoderm, but also immediately beneath the cuticle in the layers of the body-wall.

On the other hand, it may be permissible to compare these structures with something of the same kind in the Nematoids. These worms usually possess at least remnants of a coelom which is well developed, and with a limiting epithelium in *Gordius*;<sup>1</sup> in *Ascaris*, Joseph has stated ('Zool. Anz.,' Bd. v, p. 603) that it is possible to inject from the excretory pore a fine system of canaliculi lying between the muscle-cells, and in fact pervading the body generally, while Schneider (Mono-graph Nematoden) has figured the branches in *Ascaris*. In any case the canals running in the lateral lines are not a little suggestive of the longitudinal canals which I have described in *Libyodrilus*. In the *Acanthocephala* also, where there is a coelom, the lemnisci, which have been stated to occur in certain Nematoids also by Hamann, appear to be processes of the body-walls depending into that coelom; they are permeated by tubes which are connected with a system of vessels in the body-walls. These tubes may be excretory in nature, though they have been described as vascular. In any case I am not aware that a similar network of tubes has been described in the integument of any *Oligochaeta*; this worm, therefore, has

<sup>1</sup> According to Vejdosky's recent work upon the structure of *Gordius* ('Zeitschr. f. wiss. Zool.,' Bd. xliii) these Nematoids show many points of affinity with segmented worms; so much so that they should be removed altogether from *Nemathelminthes*. The excretory organs appear to be represented by coelomic canals.



an excretory system which is at least of interest in being formed on quite a novel plan.

### § Male Reproductive System.

The testes are paired structures lying in Segments 10 and 11; they are not enclosed in any sacs, and are quite independent of the sperm-sacs. The gonads are remarkable on account of their great length and thinness; the extremities are frayed out, as is usually the case among earthworms, into a number of jagged processes.

Sperm-sacs.—There are two pairs of sperm-sacs attached to the anterior walls of Segments 11 and 12; each sac of each pair is quite independent of its fellow. In the living worm the sperm-sacs have a whitish appearance with the exception of the basal part, which is greyish brown. In this part of the sac the Gregarines, which appear to be parasitic in all earthworms, are chiefly lodged, and the colour is due to brown pigment deposited round their cysts. As to the histological structure of these sacs, they agree with those of other earthworms in having their cavity greatly broken up; the sperm-sacs on their first appearance are solid, except for a very small cavity which communicates with the interior of the segment in front of that in which they lie. There are no seminal reservoirs, and thus the testes, and funnels of the vasa deferentia, depend freely into the coelom.

Sperm Ducts and Funnels.—In all the members of the family Eudrilidæ (excluding from this family Eudriloides and Pygmæodrilus) with the exception of Nemertodrilus griseus, and perhaps Preussia siphonochæta, the sperm ducts are dilated before their connection with the funnel. In Libyodrilus there is no such widening of the sperm ducts either in the neighbourhood of the funnels or anywhere else. The funnels themselves occupy the usual position in Segments 10 and 11; those of Segment 11 are more nearly opposite to the sperm-sacs than to the testes; the sperm ducts are narrow tubes of the usual appearance, but are unusual in passing through the segments which they traverse at some distance

from the body-wall; their course is straight without any windings; the two sperm ducts of one side appear to unite just behind the intersegmental septum 12—13; as a matter of fact they do not unite here or anywhere else until at their actual orifice; they lie, however, in close contact. In transverse sections the sperm ducts are seen to have quite the ordinary structure; there is no development of muscular fibres round them such as occurs in *Eudrilus*. The funnels are large and their walls are much folded.

The atria have the tubular form which is characteristic of the family; but they are short, and usually contained entirely within the 18th segment. Towards the external aperture they become narrower. These organs have the "nacreous" appearance of the corresponding organs in *Eudrilus*, which is due in both cases to the great development of the muscular coat. This reaches an extraordinary thickness in *Libyodrilus*, which is thus remarkable even among the *Eudrilidæ*.

The external orifice is single, and is situated upon the summit of a rounded elevation occupying the middle of the ventral surface of the body and extending over a portion of Segments 17 and 18. The epithelium which covers this elevation is furnished with numerous glandular cells, conspicuous on account of their dark staining. With each atrium is connected a sac in which lies a single short penial seta. The sac communicates with the distal part of the atrium that is embedded in the thickness of the body-wall.

The penial seta is illustrated in fig. 8; it is remarkable on account of its shortness and its blunt rounded free extremity, which is not ornamented.

The atrium is lined throughout with epithelium, which is separable into two strata.

The cells, however, had not in any case (I examined the atria of three individuals) the extremely glandular appearance of the corresponding epithelium in *Eudrilus*. This may, no doubt, be owing simply to a cessation in the secretory activity in the cells.

In nearly all the *Eudrilidæ* the vasa deferentia open into the

atrium. Perrier, who was the first to make known the structure of *Eudrilus*, described the vasa deferentia as opening into a pouch, into which also opened the atrium, regarded by him as the equivalent of the prostate of other earthworms. It is so figured in his memoir (10, pl. ii, fig. 26). I myself showed that, unless we were dealing with different forms, this description was incorrect; in specimens of *Eudrilus* coming from several distinct localities I found that the vasa deferentia opened into the structure that had been termed prostate. This glandular tube, I pointed out, was itself divided into two separate tubes by a longitudinal septum not visible or hardly visible externally. Into the longer of the two tubes, at a point corresponding with the apex of the shorter compartment, open the vasa deferentia, each by its own orifice. The structure of *Eudrilus* has been recently reinvestigated by Dr. Horst (7), who gives a diagram (7, p. 6) agreeing with my figure (15, pl. xxxiii, fig. 15), and remarks that "l'appareil génital mâle est exactement conforme à la description détaillée qui M. Beddard a publiée de cet organe."

In *Teleudrilus* Rosa describes the opening of the vasa deferentia into the atrium not far from the anterior extremity of the latter, i. e. not far from their point of opening on to the exterior. The same thing occurs in *Heliodrillus* and *Hyperiodrilus*; in *Nemertodrilus* the communication between atrium and vasa deferentia takes place, as it apparently does in *Teleudrilus*, not far from the external aperture of the former.

In *Libyodrillus* the arrangement is very interesting. On a dissection of the worm I could not follow the course of the vasa deferentia beyond the point of opening of the atrium; this was due to the fact that the two tubes, which retain their distinctness though they are in very close juxtaposition, at that point perforate the muscular tissue of the atrium; they pass up, at first within the thickness of the muscular tissue, and later within the epithelium, to nearly the summit of the atrium. At this point they open into its lumen by a common orifice; the two vasa deferentia unite just at the orifice; the cilia are

not continued on to the epithelium which lines the atrium. At the orifice of the vasa deferentia they are particularly long, and project for some way into the interior of the atrium. In *Libyodrilus*, therefore, the atrium has come to be, as it is in such worms as *Nais*, a kind of continuation of the vasa deferentia. Among the *Eudrilidæ* the point of opening of the vasa deferentia gradually moves down the atrium until, in *Nemertodrilus*, it comes to be placed near to the external orifice.

This series of facts lends further support to the original suggestion of *Vejdovsky*, which was strengthened by my own observations, as to the homology between the atrium of the aquatic genera and the prostates of the terrestrial genera.

If we are to follow *Dr. Benham* (11) in objecting to this identification we shall be plunged into obvious difficulties, for *Dr. Benham* considers "that a portion of the prostate in *Perichæta*, *Eudrilus*, and other genera, in which the sperm duct and the prostate join, is probably the homologue of the atrium of *Tubifex*."

I should mention that the interior of the atrium in *Libyodrilus* is not divided by a septum, and that the lining epithelium is greatly folded; this is carried to such an extent that in longitudinal sections the lumen appears to be furnished with tubular diverticula; transverse sections are required to show that these are merely foldings of the epithelium.

### § Female Reproductive System.

The extraordinary development of cœlomic pouches in connection with the different parts of the female reproductive system is the most distinguishing character of the *Eudrilidæ*, and also occurs in the present species:

**Spermathecal Sac.**—In dissecting the species which forms the subject of the present memoir, the most conspicuous organ of the body is a large sac which lies upon the dorsal surface of the œsophagus. This structure is illustrated in fig. 1, which represents a general view of such a dissection, and



more diagrammatically in fig. 9; the latter figure shows the relations of the sac to neighbouring organs. The sac is of a brownish-yellow colour, and of an irregular elongated form; it is marked here and there by furrows, which indicate that it is capable of being extended to a much greater size; it completely covers the dorsal vessel and œsophagus, to which it is attached by a series of thread-like ligaments. The sac extends when fully developed from the anterior boundary of Segment 13 to the posterior extremity of Segment 18; it gives off on either side three diverticula; from the first pair of those diverticula arise the oviducts which pass outwards so far as already stated upon the 15th segment. Anteriorly the sac divides so as to embrace the œsophagus, round which it forms a ring; immediately beneath the œsophagus the two halves become reunited, and pass forwards and downwards in close contiguity to the septum separating Segments 12 and 13. In this region the sac is very much narrower; at the nerve-cord the sac again divides so as to surround the cord, and the unpaired duct which arises from this perineural ring opens on to the exterior in the 13th segment. The sac was found to be filled with bundles of spermatozoa of irregular shapes and sizes; a fragment of this sac taken from the living ovum and teased up upon a slide showed that it was lined with peculiar granular cells. The cells are so easily detached that it appeared as if they were free in the interior of the sac; an examination of hardened material showed that this was not so, and that the cells form a lining to the sac. The fresh cells are of an irregular, generally somewhat elongated form; occasionally they are branched; the protoplasm of the cell is very clear and transparent; in the protoplasm are a quantity of spheroidal granules, usually massed together in the widest part of the cell; the cells resemble very closely the peritoneal cells which cover the alimentary tract, but the granules in their interior are greyish instead of brownish green.

In sections of the mature spermathecal sac (see fig. 12) its walls are seen to be muscular, with a coating and lining of epithelium.

Embedded in the thickness of the muscular coat is a dense layer of tissue which stains darkly; this layer is of some thickness, but apparently homogeneous throughout; here and there, for instance, in that part of it which covers the egg-sac as shown in fig. 12, favorable sections demonstrate that it is a membrane, *el*; it is usually much bent, showing the zigzag contour illustrated in the figure; in all probability it is of the nature of elastic tissue, and permits of the easy recovery of the original dimensions of the sac after it has been unduly distended with sperm.

The lining epithelium of the sac is, as a rule, more than one cell thick; there appears usually to be an innermost layer of fairly regular smallish cells; here and there an extensive proliferation of these has taken place. The whole character of the cellular lining of the sac indicates that it is not formed as an invagination of the epidermis; nor, as will be seen later, is it. It has been said that the anterior wall of the sac passes in close relation with intersegmental septum 12—13. In longitudinal sections—which are, I may remark, much more useful for studying the structure of worms than transverse sections—the anterior wall is seen to be not merely in continuity with the septum; it is formed by the septum itself.

Ovaries.—I could not find the least trace of these organs in fully mature worms, either by dissection or in sections. That the ovaries should be evanescent structures in an earthworm is rather unexpected. They are present in young stages, where they exhibit the usual position and structure, though like the testes they are much elongated and very narrow.

Egg-sacs.—In a dissection the egg-sacs can hardly be distinguished from the great unpaired spermathecal sac in which they lie; a careful inspection shows that at the point where the oviduct arises there is a slight bulging outwards of the sac; this has a browner coloration than the rest of the walls of the sac. This, however, is no more conspicuous than is represented in fig. 9, where the spermathecal sac and the oviducts are displayed in a slightly diagrammatic form.

In transverse sections the egg-sac of each side is seen to be a spherical body lying within the spermathecal sac, and supported at one side by the wall of this sac; its cavity, however, is nowhere continuous with the cavity of the spermathecal sac; the two structures are so far perfectly independent, and appear to be as it were accidentally associated. I shall show later (on p. 574) that there are also embryological reasons for regarding the egg-sac as entirely unconnected with the great unpaired sac in which it lies. The egg-sac of all earthworms in which it has been found, with the exception of the Eudrilidæ, lies in the 14th segment, attached to the anterior wall of this segment. In *Libyodrilus* there is a septum separating the 13th from the 14th segment; but this septum does not traverse the spermathecal sac; hence the egg-sacs do not depend from any intersegmental septum in the fully mature worm.

In its microscopical structure the egg-sac presents no peculiarities of any particular interest. Its cavity is, as is usual with this organ, subdivided into numerous chambers, packed with ova and germinal cells in various stages of development. The walls of the sac are formed largely of muscular tissue with abundant nuclei; the compartments which are formed by ingrowth of this layer are also muscular, but seem to be irregularly lined with granular peritoneal cells. The relations of the egg-sac to the spermathecal sac are shown in fig. 12; it will be noticed that it projects into the interior of that sac, and in other sections, taken at a different level from the one figured, it appears to lie freely in the interior of the sac. It is, however, easy to see from the histology of the parts concerned that the egg-sac is not really within the spermathecal sac; the walls of the latter are not perforated; they are merely pushed in by the egg-sac: these points will be understood by a reference to fig. 12.

The figure shows the egg-sac just where the oviduct opens into it; two parts of the oviduct are shown; the lettering *od* is placed in the oviduct where it commences to widen out into the funnel; to the right of the figure lies a section of the narrower part of the tube. Running round the walls of the egg-

sac where it projects into the spermathecal sac is a band of elastic tissue (*el.*), which I have already spoken of in connection with the spermathecal sac; it will be noticed that this band of tissue does not belong to the egg-sac but lies outside it, thus showing that the egg-sac does not really project into the spermathecal sac through its walls; it is, therefore, unnecessary to state that there is not any communication between the internal cavities of these organs. They are perfectly independent of each other though in very close contact. The egg-sacs have, in fact, the same relation to the spermathecal sac that the sperm-sacs have in certain earthworms to the "seminal reservoir." For example, in *Dichogaster* the sperm-sacs are enclosed in large thin-walled seminal reservoirs. Bergh distinguishes these sacs in *Lumbricus* as "Samenkapseln" (= seminal reservoirs), and "Samenblasen" (= sperm-sacs). The former may, and generally do, enclose other organs, e. g. the ventral blood-vessels, and, in *Dichogaster*, the sperm-sacs themselves.

The mature ova have a thick, darkly-staining membrane, in which I could find no striæ such as occur in *Hyperiodrilus* and *Heliodrilus*.

**Oviduct.**—In describing the external characters of this worm, I have already remarked upon the abnormal position of the oviducal pores. Their position is seen in longitudinal sections to be rather less abnormal. This is due to the fact that the septum which separates Segments 14—15 is attached to the middle of the latter segment, just in front of the setæ; accordingly the oviducal pores are really situated within the fourteenth segment, reckoning by the septa and not by the external furrows; they are, however, as in *Teleudrilus*, just in the boundary line between the two segments.

In dissections of the worm (see fig. 1) the oviduct is seen to pass in a straight line without any windings from the egg-sac to the exterior; its length is considerable when compared with the oviduct of *Lumbricus*, for example; in dissections the egg-sac cannot be seen, as it is entirely enclosed by the spermathecal sac. In transverse and longitudinal sections



of the genital region of the worm the oviduct is seen to be ensheathed in a tolerably thick muscular coat, as is the case with all other Eudrilidæ. In the young the oviduct is connected both with the interior of Segment 13 and with the egg-sac in the way that is generally found in earthworms. In the adult worm the large spermathecal sac nearly fills the 13th segment and encloses the egg-sac, though, as has been already said, there is no communication between the two. Near to its opening into the egg-sac the oviduct is invested with a specially thick muscular coat; it opens into the interior of the egg-sac by a not very extensive funnel; just before its opening into the egg-sac it gives off a narrow branch which passes into the wall of the spermathecal sac, becoming narrower as it proceeds, and ultimately opens into the interior of the sac by a very small aperture. The aperture into the spermathecal sac cannot be of any functional importance, since the ovary is an evanescent organ, and there are no ova to be found in the sac of the adult and nearly adult worm. At the point where the branch of the oviduct opens into the spermathecal sac the walls of the latter are specially thickened, and project into its interior in the form of a rounded pad. The formation of two openings from the originally single aperture is, of course, due to the growth of this spermathecal pouch, which cuts off the opening of the egg-sac into the body-cavity, and at the same time that part of the oviducal funnel which opened in the younger stages into the egg-sac.

#### V. Description of some Young Stages.

The youngest individual which I examined was rather more than half an inch in length. I imagine that it could not have long escaped from the cocoon.

It is quite evident from an examination of this embryo that the present species is hatched in a much more imperfect condition than either *Lumbricus* or *Acanthodrilus*, the only two genera of whose development we have at present any knowledge. In the embryo of *Lumbricus*, according to Bergh, the testes and ovaries are present while it is still within

the cocoon; in the New Zealand *Acanthodrilus multiporus*, whose development I propose to treat of in another paper, not only the gonads but the funnels of the sperm ducts and oviducts are fully recognisable in advanced embryos extracted from the cocoon.

I could not find any trace of the gonads in the young *Libyodrilus*. This may not be enough to prove that they are not present in the stage which I examined; but it seems unlikely that they would have been overlooked had they reached anything like the development that these organs have reached in the corresponding stages of *Lumbricus* and *Acanthodrilus*.

The gonads being absent, or at most very slightly developed, it is not surprising that there is no trace whatever to be found of this duct or of the terminal apparatus with which the ducts are connected. All these structures then appear to be later developments than in *Acanthodrilus* or *Lumbricus*.

One of the most striking features in sections of the embryos of *Acanthodrilus* is the enormous quantity of perivisceral corpuscles present; they are so numerous as to occupy the greater part of the coelom. The amount of the perivisceral corpuscles in *Libyodrilus* is not at all large. M. d'Udekem (12, p. 31) has commented upon the great abundance of perivisceral corpuscles in the young *Tubifex* just escaped from the cocoon.

The immature condition of this stage was also shown by the minute structure of the alimentary canal and by its contents. I thought it possible that I might detect some trace of the pouches connected with the oesophagus, which are so important and characteristic a feature of most Eudrilidæ; but there was not the remotest trace visible; the three gizzards were just commencing to be formed, but they had not acquired anything like the proportions which they ultimately assume. In describing the structure of these organs in the adult worm I have mentioned that they lie in three consecutive segments, and are separated from each other by a short, soft-walled segment of gut. These tracts (cf. fig. 1) are of very much less extent longitudinally than the gizzards themselves; in the youngest

worm (fig. 18) the reverse is the case ; the interspaces between the gizzard are very much longer than, about three times as long as, the gizzards.

The intestine is lined by a columnar epithelium, the cells of which are still loaded with spherical granules ; they resemble, in fact, the cells of the intestine in the advanced fœtus of *Acanthodrilus*. The interior of the intestine seems to indicate that the young worm had not yet commenced to swallow the soil in which it was found ; the intestine contained a granular substance, which presented the appearance of a coagulated albuminous fluid ; in this were embedded a large number of setæ of small size ; the structure of the setæ showed that they probably belonged to the same species ; at least there was nothing to indicate that they did not. So far as my experience at present allows me to say, it is not possible to distinguish the setæ of the *Eudrilidæ* from those of the *Lumbricidæ*, and, indeed, of most other groups of earthworms. The question is, How did these setæ get into the intestine of the young worm ? I noticed in the body-cavity of the same individual a few setæ embedded among the peri-intestinal cœlomic cells ; these were evidently setæ of the same individual that had become freed into the body-cavity ; if they can work their way through the intestinal epithelium we have an explanation of how they come to be found in the cavity of the intestine. It seems more probable, however, that they were originally swallowed, and in this case it appears likely that the cocoon contains up to a comparatively late period more than one developed embryo ; the individual or individuals which succeed in reaching the full term of their development must feed upon the others when alive or upon their dead bodies.

The structure of the gizzard in longitudinal section is shown in fig. 18. The circular muscles appear to be derived from columnar cells, the nuclei of which are shown ; whether these cells are produced by the division of the hypoblastic cells, or from the splanchnopleure, which is most probable, I am unable to say. The muscular fibres themselves are arranged in a



more or less regular fashion along both sides of those cells. The typhlosole is a simple fold.

The integument in the youngest specimens at my disposal was chiefly interesting on account of the structure of the epidermis and of the longitudinal muscular coat. In the epidermis the gland-cells are limited to an area in the middle of each segment ; it is evidently here that they first appear. In the longitudinal muscular coat the fibres are embedded in the same gelatinous material recognisable in mature worms, but this is proportionately less in amount, and there is no thick layer of it separating the longitudinal muscles from the peritoneum. The fibres themselves approximate in their arrangement very closely to *Lumbricus* ; that is to say, they are disposed in double columns, which are not, however, perfectly regular.

The nephridia in these embryos presented features of considerable interest. The paired nephridia were recognisable in all the segments after, and including, the fourth ; the first pairs are not covered with the coating of peritoneal cells containing numerous spherules, as are the succeeding pairs. This investment of the nephridia commences gradually, inasmuch as those of the fifth pair had only a very slightly developed peritoneal layer, in which, however, the granules of secretion, which stain deeply, were perfectly plain.

The anterior pairs of nephridia are, as also in the adult worm, closely attached to the posterior wall of their segment. They are not bound thereto by any mesentery, but lie in actual contact with the septum. The duct leading to the exterior passes down, in the case of the anterior nephridia at any rate, also in close contact with the septum ; it is much coiled, and so in longitudinal sections appeared, in every section, cut at right angles to its course. Arrived near to the insertion of the septum on to the body-wall the duct joined a continuous longitudinal duct, passing along the first few segments of the body and putting the nephridia of these segments into communication with each other. On each side of the nerve-cord this duct was found having similar relations to the nephridia of the segments through which



it passes. The duct is not embedded in the longitudinal muscular coat or in the peritoneum, but appears to lie in the coelom.

If this duct had turned up two or three years earlier than it has, I should have been able to claim it as a discovery of some importance. The account given by Balfour of Hatchek's statements about the developing nephridia of *Criodrilus* gave additional strength to them, although Balfour considered them to need confirmation, and the apparent confirmation which is given by a young, only just out, foetal earthworm would have almost clinched the matter. The discovery even of Meyer and Cunningham that the adult *Terebella conchilega* is furnished with such a longitudinal duct was regarded of the greatest importance in that connection. As it is, the new fact recorded here has lost a good deal of importance by appearing too late. Since Hatchek's paper the aspect of the nephridial question has changed. Nevertheless it is of some little importance. In *Perichæta* there does not appear to be, in the adult at any rate, a pair of distinct longitudinal ducts passing from segment to segment; there is simply the irregular network of each segment which is connected here and there with that of adjoining segments. Spencer, however, has described (9) in *Megascolides australis* a pair of longitudinal ducts which run from segment to segment, putting the complicated nephridial network and the large paired nephridia of successive segments into direct communication. It is not yet known how early these ducts appear in *Megascolides*, but it is interesting to notice that they appear at least comparatively early in the development of *Libyodrilus*. In the adult worm they persist (fig. 7), but their importance is lost in the complications of integumental network. For all that I know the paired longitudinal ducts may be the first of the nephridial system to appear. I very much regret that my material does not enable me to settle this question. At any rate, it is certain that in *Libyodrilus* the integumental network connected with the paired nephridia is preceded by paired longitudinal ducts uniting those nephridia.

In the stage that I am describing here, there was no discoverable trace of the integumental network; the external pores of the nephridia were apparently paired, but as they were very minute, I may possibly have overlooked other orifices. It should be mentioned that the nephridial funnels exist at this stage; they lie in a plane considerably above that in which the longitudinal ducts lie, and there is, therefore, no chance of my having confounded the longitudinal duct with the tube leading to the funnel.

**Generative Organs.**—In a specimen without a clitellum, but as large or nearly so as some specimens with a clitellum, the spermatheca was not visible from the exterior; that is to say, there were no traces that could be seen, even with a lens, of the external pore, which, though small, is quite evident in mature or nearly mature specimens.

Longitudinal sections revealed a very remarkable condition of the female reproductive apparatus.

The ovaries, receptacula ovarum, and oviducts present the same arrangement as in more typical genera of earthworms (*Lumbricus*, for example).

The epidermis in the middle of Segment 13 for a small space is different from the rest; this difference, however, merely consists in the entire absence of glandular cells. This modification of the epidermis, which is illustrated in fig. 13, first appears in this stage; it is not directly inherited from the epidermis of the embryo, as yet undifferentiated into gland-cells and packing-cells. In the earlier stages which I have already described there is no means of distinguishing by the characters of the epidermis the median part of Segment 13, which is so marked in the present stage. A complete series of sections through the 13th and neighbouring segments showed a small sac (fig. 13) lying immediately beneath the nerve-cord and in contact with the body-walls. The walls of this sac are chiefly muscular; it is lined with a layer of cells, of which the nuclei alone were darkly stained; the outlines of the cells themselves were rather hard to make out, but the epithelium was evidently composed of very low

cells; these cells had the appearance rather of peritoneal cells than of invaginated epidermic cells.

The sac, where it lay beneath the nerve-cord, had a flattened appearance, as if it had been squeezed between the nerve-cord and the body-wall; its cavity was chiefly developed in a horizontal, not in a vertical, direction. It could be traced round the nerve-cord for a short way on each side as a narrowish tube running forwards and backwards in close contact to the thinnish septum which divides the 13th from the 12th segment; coming into close relation with the base of the ovary, it ended in the neighbourhood of this organ.

Traced in the other direction the sac penetrated the longitudinal muscular coat for a short distance, and gave off a narrow tube, ending blindly some way below the circular muscular layer: the space between the ends of this tube and the epidermis was crowded with nuclei, but there was no break that I could find in the circular layer of muscles, and no invagination of the epiblast. The sac is thus entirely closed; it has no communication with the exterior, which might have been expected.

The opposite extremity of the sac does not form a closed sac, as indicated in the figure; it appears to do so in some sections, such as the one illustrated in my drawing; a complete series of sections shows that this sac communicates freely with the body-cavity. One wall is formed by the septum which separates Segments 13/14, lettered *spt*; the opposite wall is thinner, and consists of a membrane, which connects that septum with the one behind; the membrane in question is, however, not attached to the intersegmental Septum 13/14 for the whole of its extent; it thus forms an aperture which leads into the subneural pouch.

In specimens rather more immature than the one just described, the relations of the subneural pouch will be understood by a reference to figs. 4—6, which represent three sections of a continuous series; those selected for illustration represent the critical points in the series; the first one (fig. 4)

shows the continuity between the subneural pouch and the body-cavity; the walls are seen to be formed from the intersegmental Septum 13/14, and form a membrane (*S*), which, traced backwards, comes into continuity with intersegmental Septum 14/15. In the second section (fig. 5) the two membranes join above the subneural pouch; the third section (fig. 6) is through the ventral median line, and the nerve-cord is, therefore, indicated. The subneural pouch is seen to lie below the nerve-cord, and in contact with the longitudinal muscular coat of the integument; the intersegmental Septum 13/14 does not reach the nerve-cord, and to its free extremity is attached the membrane uniting it with the following intersegmental septum. In this specimen the epidermis lying immediately below the subneural pouch is exactly like the epidermis elsewhere, i. e. the glandular cells are not absent. It will, I hope, be clear from the above description and from the figures that the spermathecal sac is first of all formed by certain membranes in the coelom, which become approximated and attached in parts to form a sac. Later on the sac burrows its way towards the exterior, where it appears to be met by a very limited invagination of the epidermis. This mode of formation has a remarkable analogy to the formation of the oviducts in certain ganoid and teleostean fishes. It is very probable that, as I have already suggested, the second oviduct in *Eudrilus* is formed in a similar way. It will be observed that in early stages the ovary comes to lie within the spermathecal sac. If, for some reason or other, the backward extension of the sac were checked, it would form simply a duct for the ova.

There is also an interesting analogy to the formation of the nephridia and genital ducts in *Peripatus*, which have been recently so successfully investigated by Mr. Sedgwick.

In a species of *Moniligaster*—*M. Houteni*—Dr. Horst has described the oviduct in a way which suggests that it resembles the early stage of the spermathecal sac of *Libyodrilus*. His description (13, p. 99) is as follows:—"As stated before, the 12th and 13th septa are placed close against each other; these two septa seem to form together on



each side a sort of funnel, the inferior part of which communicates with one of the pores on the 14th ring. Although the ovaries could not be found, I suppose that this funnel may function as an oviduct."

In *Nemertodrilus* the spermathecal sac is in a condition of arrested development; it resembles to a certain extent the early stages in the development of *Libyodrilus*, but secondary modifications—in the complicated fringe which surrounds the pores on Segment 13—appear to prevent their performing the function of an oviduct. It would be very interesting to have some information about the development of the large sacs in *Nemertodrilus*, particularly as to whether they originally communicate with the egg-sacs or not.

## VI. Homologies of the Reproductive Organs in *Eudrilidæ*.

The homologies of the different parts of the reproductive system in *Eudrilidæ* are by no means easy to decipher.

It is possible, however, to attack the problems with greater confidence in view of the developmental facts that I have been able to bring forward. Hitherto nothing but the adult structure was known, and that in certain cases evidently imperfectly. It is clear that in many points the *Eudrilidæ* differ greatly from all other earthworms; and I think that this paper rather accentuates the differences than removes them.

On the other hand, I have been able to show that at a certain stage of development the female reproductive system closely resembles that of more normal genera; the special peculiarities of the *Eudrilidæ* are a later formation; this argues for the isolated position of the group, which I have elsewhere urged, but not for its primitive characters; the reproductive system must be looked upon as much modified from that of other earthworms.

It seems fairly clear that the large unpaired sacs which exist in the genera *Heliodrilus*, *Hyperiodrilus*, *Stuhlmannia*, *Polytoreutus*, *Paradrilus*, *Preussia*, *Nemertodrilus*, and *Libyodrilus* correspond in every case. But they are in

this event not spermathecæ, except in function. A true spermatheca, as I have shown, exists in *Heliodrillus* and *Hyperiodrilus*, partly or entirely enclosed by the median sac. In *Paradrillus*, too, judging from Michaelsen's figure, there is a spermatheca of this kind, but apparently not in some of the other genera; nor is this structure represented in *Libyodrillus*. In *Libyodrillus* the large unpaired sac is obviously a spermatheca in function, for I found sperm in it, but its development shows that it cannot possibly be compared to the spermatheca of, e. g., *Lumbricus*. It is a mesoblastic pouch which has acquired an external opening; whereas the spermatheca of *Lumbricus* are known, through the investigation of Bergh, to be purely epidermic involutions. The homologue of the spermatheca of *Heliodrillus* and *Hyperiodrilus* in *Libyodrillus* appears to be the orifice only of the mesoblastic sac. In this connection the structure of *Nemertodrillus* as described by Michaelsen is of great interest. He has described (3), and I have been able (1) to entirely confirm his description, a pair of apertures in Segment 13 which lead into the interior of that segment; they have no connections with the sacs extending backwards from Septum 13—14, but Michaelsen has suggested that they represent the orifices of those sacs; I am now of opinion that this suggestion is perfectly correct. So far *Nemertodrillus* appears to be a degenerate form, and other structural facts confirm that view. It is a little difficult to understand the arrangement of the various organs in *Preussia*, since Dr. Michaelsen was unable, through being obliged to respect museum specimens, to make out the relation of the ovaries.

The following is Michaelsen's description of the parts in question: I think it worth while to quote it in full, as the journal in which it is contained may not be accessible everywhere.

“Der weibliche Geschlechtsapparat zeigt wieder eine neue Modifikation der eigenartigen Verwachsung, wie sie für die *Teleudrilien* charakteristisch ist. Die ventral median Öffnung

im 15 Segment führt in eine lange, gestreckt birnförmige Tasche, die sich bei dem untersuchten Exemplar bis in das 19 Segment nach hinten erstreckt. Nach vorne schien der Stiel dieser Tasche in zwei grosse, ovale, dünnhäutige Blasen ueberzugehen, die fast bis an den Anfang des 13 Segments reichen. In dem nach hinten gerichteten Pole dieser Blasen münden die Eileiter ein. Diese gehen von ihrer Ausmündung seitlich am 14 Segment, zuerst in grader, senkrechter Richtung auf die Medianebene zu. Weit bevor sie dieselbe erreichen biegen sie nach hinten um. Zugleich verdicken sie sich bedeutend. Das Dissepiment 14/15 durchbrechend, verlaufen sie nach hinten bis vor das Dissepiment 15/16, biegen sie dann wieder nach vorne um und münden schliesslich in die erwähnten Blasen ein . . . . . jeder Eileiter trägt an der ersten Knickung, an der Übergangsstelle von dem engen distalen Teil zu der erweiterten, nach hinten gerichteten Schleife, ein verhältnissmässig lang gestieltes Receptaculum Ovarum."

It appears to me that the "thin-walled vessels" and the tube leading to the receptaculum correspond to the first of the diverticula of the median sac in *Libyodrilus*, and that the distal extremity of the "pear-shaped pouch" is the true spermatheca. It is rather harder to understand *Eudrilus* and *Teleudrilus*. The former genus has been redescribed by Horst (7), his description of figures being confirmatory of my own. There seems, therefore, to be little doubt, now that so careful a worker as Dr. Horst has looked into the matter, that the adult structure of the female genitalia of *Eudrilus* is known.

I must, in the first place, admit that the structure of other *Eudrilids* is not confirmatory of my view that in *Eudrilus* there are two pairs of ovaries and two pairs of oviducts. I do not, however, admit that that view is yet entirely disproved. In order to show that the tube, which I have regarded as the oviduct of Segment 13, is not an oviduct, it must be shown that the organ regarded by Perrier, Rosa, Horst, and myself as a spermatheca is a cœlomic sac comparable to that of *Hyperiodrilus*, &c. I am inclined, however, to believe that this will

be proved ; but then it will have to be farther proved that the duct of that sac up to a point beyond the "oviducal" orifice is also derived from the cœlomic sac. Now, Horst says (7, p. 232) that the muscular layer of the spermatheca becomes very thick near to the point of opening of the ovarian duct (= my "oviduct of Segment 13"). This looks as if the true spermathecal invagination began, or rather ended, at this point. If so, it would be reasonable to speak of the tube in question as an oviduct, whereas if it were merely a portion of the cœlomic sac it could hardly be termed an oviduct, but rather an extension of the said sac forwards.

It is clear that two pairs of ovaries and oviducts formerly existed in earthworms ; not only do they occasionally occur as a sport (see my remarks upon varieties of *Perionyx*), but they occur normally in *Phreoryctes*. Considerable traces of the missing parts are met with in embryos of *Lumbricus* and *Acanthodrilus*. Dr. Horst has overlooked these facts, some of which were known when he wrote (7, p. 239), "*Chez tous les genres d'Oligochètes, on n'a trouvé jusqu'ici qu'une paire d'ovaires.*" To return to the immediate subject of discussion, it seems probable that the large sacs termed spermathecae in *Eudrilus* and *Teleudrilus* correspond to the cœlomic pouches of *Libyodrilus* and the other forms. The question is how much of them corresponds ; it may probably be safely assumed that up to the point of opening of the oviducts there has been an epidermal invagination ; but beyond that inference I do not consider it safe to go at present.

## VII. Definition of Genus and Species.

### Genus *Libyodrilus*, F. E. B.<sup>1</sup>

Nephridia paired, but connected with a network of tubes ramifying in the integument ; those of some of genital segments disappear in mature worms, but network remains. A large unpaired sac opening on Segment 13 extends through

<sup>1</sup> I have briefly characterised the species in 'Proc. Zool. Soc.,' 1891, p. 172.



five segments and lodges receptacula ovarum; oviducts pass from these to apertures on 15th segment. Atria two, with thick muscular walls opening by a common orifice on middle line between Segments 17, 18. Each is furnished with a single penial seta. Vasa deferentia without dilatations or muscular coat, open near to summit of atria. Œsophagus without calciferous glands or ventral pouches; three gizzards present at end of œsophagus in Segments 23, 24, and 25. Integument without sense bodies.

#### *Libyodrilus violaceus*, F. E. B.

Setæ strictly paired and ventral in position. Penial setæ short with a rounded free extremity. Clitellum occupying three segments (14—16). Dorsal vessel single. Colour dark violet with a tinge of pink.

Habitat—Lagos, West Africa.

The above definitions must in the present state of our knowledge be regarded as only tentative.

### VIII. Summary.

The principal new facts in this paper may be briefly summarised as follows:

(1) The nephridial system consists of paired nephridia which do not open immediately on to the exterior, but are connected with an extensively ramifying system of tubes embedded in the circular and longitudinal muscular coats; these tubes consist of four principal longitudinal trunks continuous from segment to segment, and of a single large circular vessel in each segment passing right round the worm at the junction of the circular and longitudinal muscles; these are connected by a plexus of vessels, and numerous tubules, leading to the exterior, are given off from the circular trunk. In some of the genital segments the paired nephridia have almost disappeared, leaving only the integumental network. Nothing of the kind has been yet described in any *Oligochæte*. In the young worm, just escaped from the cocoon, there is no

integumental network, which must, therefore, be regarded as secondary, but the anterior nephridia at any rate are connected on each side by a continuous longitudinal duct lying within the cœlom.

(2) In the young worm the reproductive organs agree with these organs in other earthworms; in the adult, a large unpaired sac lying over the gut is developed; this sac encloses the receptacula ovarum, and opens by a median pore on Segment 13. It is developed from mesoblastic tissues, and is not therefore the morphological equivalent of the spermathecæ in *Lumbricus*, &c., but it performs the same function; the sac is formed internally and then grows out towards the epidermis; it is at first in open communication with the cœlom; its front wall is formed out of the intersegmental septum between Segments 12, 13; the ovaries are enclosed by it, but disappear early, before the sac is completed; otherwise the ova would be probably unable to enter the egg-sac which becomes nearly completely shut off from the sac; the two are in communication only by the oviducal funnel, which has become divided by the growth of the spermathecal sac into two separate tubes, one opening into the spermathecal sac, the other into the closed egg-sac; they unite, of course, to form the oviduct itself, which opens on to the 15th segment, reckoning by the external furrow, but on to the border line between Segments 14, 15, reckoning by the septa.

(3) The testes and the vas deferens funnels are quite typical in their structure and position; so, too, are the (two) pairs of sperm-sacs (in Segments 11, 12). The sperm ducts are not, as they are in other Eudrilidæ, dilated to form sperm reservoirs; they open into tubular atria, with thick muscular walls and glandular lining, near to their blind extremities; the two atria open by a common pore upon the border line between Segments 17, 18; each is furnished with a short penial seta not ornamented.

(4) The alimentary tract has no calciferous glands or ventral œsophageal pouches such as are found in other Eudrilidæ; at the end of the œsophagus are three gizzards, one to a

segment; the intestine which immediately follows has at first three typhlosolar folds; later on the two lateral and shorter folds disappear. The ventral wall of the pharynx is connected with the nephridial tubes of its segments; they open into the interior of the pharynx.

(5) The area surrounding the setæ of each side of the body is shut off from the general body-cavity, forming a paired series of chambers; in the œsophageal region is developed a perihæmal cœlomic space surrounding the subœsophageal vessels.

## EXPLANATION OF PLATES XXXVIII & XXXIX,

Illustrating Mr. Frank E. Beddard's paper "On the Structure of an Earthworm allied to *Nemertodrilus*, Mich., with Observations on the Post-embryonic Development of Certain Organs."

### ANATOMY OF *LIBYODRILUS VIOLACEUS*, NOV. GEN., N. SP.

FIG. 1.—Dissection to show the principal organs. *t.* Testes. *Sp. s.* Spermathecal sacs. *od.* Oviduct. *v. d.* Vasa deferentia. *at.* Atrium. *S.* Spermathecal sac. *g.* Gizzard.

FIG. 2.—Ventral surface of Segments 13—18. *sp.* Spermathecal pore. ♀. Oviducal pores. ♂. Male pore.

FIG. 3.—Fragment of cubicle, showing nephridial pores (*n.*).

FIGS. 4, 5, and 6.—Three figures drawn from a continuous series of sections, and illustrating the development of the spermathecal sac. *Spt.* Septum separating Segments 12, 13. *S.* Hinder wall of spermathecal sac, the cavity of which is lettered *Sp. sac.* *ov.* Ovary. *N.* Nerve-cord.

FIG. 7.—Longitudinal section through a portion of one of longitudinal nephridial ducts. *a.* Branches given off from duct. *N.* Junction with one of paired nephridia. The thick gelatinous connective tissue in which the duct is shown; *n.* Nuclei of this tissue.

FIG. 8.—Penial setæ.

FIG. 9.—Spermathecal sac and neighbouring organs. *S.* Sac. *a.* Diverticula of the same. *r. o.* Receptaculum ovarum. *od.* Oviduct. *œs.* Œsophagus. *n.* Nerve-cord.

FIG. 10.—Section through a portion of one of paired nephridia. *a, b.* Nephridial tubes. *n.* Nucleus of same. *c, c'.* Peritoneal investment.

FIG. 11.—Three nephridia of posterior segments on one side of body, as seen on a dissection. *n*. Nerve-cord. *Spt*. Intersegmental septum. *nph*. Nephridium. *f*. Funnel. *S*. Setæ of ventral pair.

FIG. 12.—Transverse section through receptaculum ovarum and spermathecal sac. *Sp. sac*. Spermathecal sac. *ov*. Ova. *od*. Oviduct. *el*. Elastic membrane surrounding sac.

FIG. 13.—Spermathecal sac at a later stage of development than Figs. 4—6, illustrated by a transverse section through body-wall. *Ep*. Epidermis. *m*. Circular muscles. *m'*. Longitudinal muscles, with lymph spaces (*l*). *N*. Nephridial tube. *Sp. sac.*, *Sp. sac*<sup>2</sup>. Two portions of spermathecal sac. *Spt*. Intersegmental septum 12—13. *ov*. Ovary.

FIG. 14.—Transverse section through body-wall, to show the ramifications of the excretory tubes. *ep*. Epidermis. *m*. Transverse muscular layer. *m'*. Longitudinal muscular layer. *Bl*. Blood-vessels. *d*. Longitudinal nephridial vessels. *d'*. Smaller tubes, forming network among the muscles. *a*. Junction with one of paired nephridia. *c*. Circular nephridial vessel. *l*. Tube running to external orifice.

FIG. 15.—Section through ventral wall of pharynx, to show opening of nephridium. *o*. Orifice of nephridium. *ner*. Nerve in pharyngeal epithelium. *m*. Muscles. *nph*. Nephridial tubes.

FIG. 16.—Diagrammatic transverse section of body, to show integumental nephridial network. *D. v*. Dorsal blood-vessel. *S. I*. Supra-intestinal blood-vessel. *I. I*. Infra-intestinal vessels. *v*. Ventral blood-vessel. *Nph*. Nephridium. *l*. Longitudinal trunks (four in number) of integumental network. *c*. Circular trunk lying between circular and longitudinal muscles. *a*. Circular vessel lying beneath longitudinal muscular layer of body-wall.

FIG. 17.—Transverse section to show perihæmal space surrounding infra-œsophageal blood-vessels (*Bl. vess.*). *œs*. Œsophagus. *corp*. Perivisceral corpuscles. *mes*. Walls of perihæmal space. *m*. Muscular bands.

FIG. 18.—Longitudinal section through one of the three gizzards in a very young specimen. *giz*. Epithelium of gizzard. *Per*. Peritoneum. *Spt*. Septum.

FIG. 19.—Transverse section through body-wall, more diagrammatic and less highly magnified than Fig. 14. *S*. Setæ sac. *Bl*. Blood-vessel. *M*. Muscle, connecting setæ of lateral and ventral couples. *Cœl*. Cœlomic space cut off from general cavity of segment by membrane *Sp*. *N*. Nephridial tube. *L*. Longitudinal vessel of integumental nephridial network; the network is also indicated in white.

FIG. 20.—Highly magnified transverse section to show circular nephridial vessel (*n*). *m*. Nerve accompanying it. *L. m*. Longitudinal muscles. *t. m*. Transverse muscles.

FIG. 21.—Infra-œsophageal blood-vessels in Segments 9, 10, 11. *a*. Branches of the same. *Sp. sac*. Sperm-sacs.



## Some Points in the Development of *Scorpio fulvipes*.

By

**Malcolm Laurie, B.Sc., F.L.S.**

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With Plate XL.

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IN the introduction to my paper on the development of *Euscorpius italicus* ('Quart. Journ. Micr. Sci.,' vol. xxxi) I mentioned that a species of scorpion (*Scorpio fulvipes*), which I was permitted to examine by the kindness of Professor Lankester, showed a considerable difference in its mode of development from that of *Euscorpius*. This difference is, as there stated, fundamentally due to the absence of yolk from the egg of *Scorpio fulvipes*, in which particular it contrasts strongly with that of *Euscorpius*, in which the proportion of yolk is enormous. Further examination of more abundant material, which Professor Bourne of Madras collected and preserved with great care and forwarded to Professor Lankester, shows that the specialisation of the embryo in relation to its mode of nutrition has reached a very high pitch.

A few notes with regard to this particular form of *Scorpio* development will, it is hoped, be of some service towards an understanding of the embryology of Scorpions, and Arthropoda generally.

### Ovary and Ovarian Egg.

The species of Scorpion of which the female reproductive organs are described by Duvernoy appears to agree in the pecu-

liarities of those organs with *S. fulvipes*. Duvernoy, however, deals only with the external appearance of ovaries in which the development of the embryo had already advanced some way.

In *Scorpio fulvipes* the ovary agrees with that of *Euscorpius* in its anatomy, in so far as it consists of a network of tubes bearing a number of eggs which cause the tubes to bulge at intervals into the body space. Beyond this, however, there are many important differences. The ovarian tube in place of the large oval sessile ova of *Euscorpius* bears a number of long diverticula, each of which ends in a solid coiled appendix (fig. 1). These diverticula were described by Duvernoy<sup>1</sup> as the eggs, but the comparatively small egg occupies only one tenth of the length of the diverticulum, and lies at its distal end at the root of the appendix (fig. 1, *ov.*).

The formation of these diverticula can be easily understood from fig. 2, which shows a section of a young egg such as *ov'* in fig. 1. The egg, which is formed as in *Euscorpius* by the growth of one of the cells of the inner layer of the two-layered ovarian tube, is carried out at the end of an outgrowth of the ovarian tube, and is at first completely surrounded by a mass of cells of the inner layer. At a stage somewhat later than that in fig. 2 an opening is formed through these cells, so that the ovum is only separated from the cavity of the diverticulum by the vitelline membrane which surrounds it. There is no specialisation of any of the cells for the purpose of forming yolk, and in fact the egg is, when ripe, entirely without food yolk.

The whole diverticulum, as is seen in fig. 1, consists of four distinct regions:—A long stalk (*st*), a thickened collar (*c*), a somewhat conical portion in which the ovum lies (*ov*), and a long appendix. The stalk is peculiar in that the continuation of the inner layer of the ovarian tube is divided into two distinct portions. The inner one (fig. 3, *i.l.*) next the lumen is formed of a single layer of long cylindrical cells with their

<sup>1</sup> Duvernoy, "Fragments sur les organes de la Génération de divers animaux," 'Mém. de l'Acad. Sci. de l'Institut,' t. xxiii.

nuclei at various levels, giving at first sight the appearance of a folding of the walls. The outer portion (fig. 3, *i.l.*) is composed of a closely packed mass of small cells of which only the nuclei can be made out. Outside this outer portion is the continuation of the outer layer of the ovarian tube (*o.l.*), which consists of flattened cells.

In the collar (fig. 4) the two portions of the inner layer are no longer distinguishable. The whole layer, which is enormously thickened, consists of clear, highly refracting cells with oval nuclei and well-marked outlines. As development proceeds the collar gradually moves down towards the ovarian tube, and as it passes the cells of the diverticulum change their form and come to resemble those of the collar. This change is preparatory to the active excretion of nutritious matter for the nourishment of the embryo in its earlier stages. The greater thickness of the collar is simply the sign of the activity of the cells within, which require more space in which to undergo their change in form. When this change is accomplished the diverticulum returns to its former dimensions.

The conical portion above the collar (fig. 1, *ov*) consists of the egg, which when ripe is less than .2 mm. in length (that of *Euscorpius* measuring 1.5 mm.), and of the inner layer cells surrounding it. This follicle is several cells thick, the cells being the same in appearance as those already described in the collar. Between the egg and the lumen of the diverticulum is a small passage, the cells surrounding which are long, and have their nuclei at their outer ends. These three proximal portions of the diverticulum together reach a length of about 2.5 mm.

The coiled appendix, which is longer than all the rest of the diverticulum, consists of a solid rod of cells (fig. 5, *i.l.*) forming a continuation of the inner layer, and is surrounded by the flattened cells which represent the outer layer of the ovarian tube (*o.l.*). The appendix plays an important part in the nutrition of the embryo, being gradually absorbed as development proceeds.

The embryo undergoes the whole of its development in the diverticulum, and does not pass into the ovarian tube until it is ready to be born.

The development extends over more than six months, my earliest stages having been preserved towards the end of October and the latest in May. How much longer it may take I cannot say, but the oldest embryos which I have examined are still some way from being fully developed.

#### Development of the Embryo.

In the earliest stage examined by me the ovum is completely segmented. The external appearance of the diverticulum and appendix is shown in fig. 1, and has been described above. A transverse section (fig. 6) shows the ovum, which is about  $\cdot 12$  mm. in diameter, to consist of an irregular mass of cells with faintly marked outlines and large oval nuclei. There appear to be spaces between the cells, but this may very likely be due to shrinkage. The nuclei for the most part stain very faintly with carmine, and show a slightly granular structure. Here and there one of the nuclei stains very darkly, and such nuclei appear to be undergoing division. The nuclei are scattered about irregularly, and show no tendency to the formation of layers. There is no trace of yolk either in the cells or in the spaces between them.

In the next stage, between which and the one described above there is a considerable gap, various changes have taken place. The ovum has increased in size to about  $\cdot 15$  mm. diameter, though there is considerable individual variation in this respect, and shows the beginnings of several structures. The cells are chiefly aggregated along one side—the ventral—and towards the posterior end, leaving a space in the middle which is full of finely granular substance. This space is well seen in longitudinal section (fig. 8), and a section in this direction also shows very clearly the stomodæum (figs. 7 and 8, *st.*). This structure is a tube, the walls of which are at first one cell thick (fig. 7, *st.*), and the lumen of which opens to the exterior of the embryo at the anterior end (i. e.



the end furthest from the ovarian tube), and at the posterior end into the space in the middle of the embryo described above. It is certainly extraordinary that the stomodæum should be the first structure developed, but the reason will be found in the peculiar mode of nutrition of the embryo in its later stages.

The outermost layer of the embryo (fig. 7, *am.*) is separated from the rest by a distinct space extending all round. It represents the so-called amnion, which is so well developed in *Euscorpius*, but in this form appears to be confined to the earlier stages, as I have been unable to find any trace of it in the later ones. A protective envelope of this sort is not so necessary in this form, where the embryo remains in situ till it has attained its full growth, as it is in a form like *Euscorpius*, where the eggs pass into the ovarian tube at an early stage, and its slight development and early disappearance are probably due to this change of habit in the embryo. Its presence seems to point to the condition in *Euscorpius* being the more primitive, a conclusion which is supported by many other facts in the development.

In the next stage, in which the embryo has increased very considerably in length, the body-wall (fig. 9, *ep*) is very thin, and composed of flattened cells on the dorsal and lateral surfaces; while on the ventral surface, where the mesoblast and nervous system will afterwards make their appearance, it is formed of two or three layers of cells with large round nuclei.

The gut is not yet formed, but its position is shown by a large cylindrical mass of yolk, the central portion of which has a curiously honeycombed appearance. The rest of the body space is filled with yolk spheres, the material for the formation of which must have been derived from the cells of the diverticulum.

The cavity of the diverticulum beyond the point to which the embryo extends is full of a finely granular substance secreted by the cells which line it. The granular structure may be due to coagulation of a fluid by alcohol. Here and

there, in the midst of this granular substance, may be seen the nuclei of free cells. These, probably, play some part in the preparation of the nutritious material for absorption by the embryo, but whether they are derived from the embryo or from the walls of the diverticulum, I have not been able to ascertain, though I think the latter most probable.

At the posterior end of the body there is a considerable mass of cells, the growth and multiplication of which provide for the rapid increase in length of the embryo. The head end (fig. 10) consists of an almost solid mass of cells, in the middle of which is seen the laterally-compressed lumen of the stomodæum, which has a highly refractive, probably chitinous lining.

At the sides of the stomodæum the cells have become elongated (*m*), and form masses of muscular fibres, extending from the stomodæum to the lateral walls of the embryo. A pair of solid outgrowths on the ventral surface represent the chelicerae (fig. 10, I).

No traces of the other appendages are present, and it is noteworthy that this pair of appendages, which in *Euscorpius* appears later than the five succeeding pairs, should here be the first to be formed.

In the next stage only a few points need be noted. Among the most curious of these is the formation of a series of dorsal outgrowths of the body, one to each segment, which give it, when viewed from the dorsal surface, the form shown in fig. 11, while in section it has the shape seen in fig. 12. The reason for this curious change in the shape of the body is not clear, as the spaces thus formed are unoccupied by any structures except a few thin bands of mesoblast. A section (fig. 12) shows the gut completely formed as a tube of large cells surrounding a granular mass of food material. The rest of the body-cavity is occupied only by thin trabeculae of mesoblast except on the dorsal surface, where the mesoblast is present in considerable amount and is hollowed out to form the heart. The yolk spheres, which in an earlier stage occupied the body-cavity, have been completely absorbed, and, except for a slight thickening

of the epiblast where the ganglia will be formed, there is no trace of the nervous system in the body. In the cephalic region the brain is beginning to form as in *Euscorpium* by the proliferation of the cells of a pair of cerebro-optic invaginations, which are more lateral in position than in the latter species, but otherwise completely correspond to them.

At a considerably later stage the gut begins to be constricted by both circular and longitudinal bands of mesoblast (fig. 13), which divide it into a central tubular portion with a series of large diverticula which form the so-called liver. Both the gut and the liver remain full of granular matter, from which the body-cavity is quite free. The ventral nervous system is by this time completely separated from the epiblast, but as its formation presents no special points of interest I have not described it in detail. In the head the stomodæum becomes very chitinous, and is furnished with powerful lateral muscles (fig. 14, *m*). Just opposite the aperture of the stomodæum is the lower end of the cord of cells which forms the coiled appendix described above (p. 589), and it is by the destruction of this cord of cells that the embryo nourishes itself through all the later stages of embryonic life. This mode of nutrition, which was imperfectly described by Duvernoy,<sup>1</sup> is very exceptional, and, indeed, more like the nutrition of a young marsupial than anything else. The cord is held in position by the chelicerae (fig. 14, *I*), and this is the object of the phenomenally early development of these appendages as well as of the stomodæum. The comparatively early formation of the gut is due to the same cause. As might be expected, the chelicerae are considerably modified to serve their peculiar function. This is best seen in the oldest stage in my possession (figs. 15—17), where the chelicerae, apart from their great proportional size, are seen to have the third joint specially developed (fig. 17, *I*, 3). This third joint is very much larger than the other half of the pincer, and is further provided with a strong band of chitin which runs in a somewhat sinuous manner from the base of the joint up to the tip. This band of chitin is grooved, and the

<sup>1</sup> Loc. cit.

two chelicerae are twisted so that the chitinous plates come into contact with each other, leaving a small hole (fig. 16, *co*) through which the cord of cells passes to the mouth. I think it is probable that the chelicerae do not merely hold the cord in position, but serve also to crush it. The lower end of the cord consists of horny-looking cell-walls from which all the protoplasm seems to have disappeared. Whether the embryo nourishes itself on the protoplasm of the cells or whether the cell contents are some special substance I have not been able to find out. It is perhaps worthy of remark that the chitinous plates on the chelicerae are covered in places with a scale-like marking very similar to that which is so characteristic of the fossil Merostomata. The epistomial lobe (figs. 16 and 17, *est*) is very well developed, and also furnished with chitinous plates.

The five succeeding pairs of appendages closely resemble their adult state, and need no description here.

The genital opercula are in my latest stage not yet visible in a surface view, contrasting in this respect with the pectines (fig. 15, *vii*).

The six metasomatic or caudal segments present one point of interest in *Sc. fulvipes*, namely, that the tail is bent up over the back, contrasting in this respect with *Euscorpis*, in which the tail lies along the ventral surface.

From what I was able to make out as to the development of the median eye, I think that the pigment-cells are epiblastic. The retina of the eye is formed, as in *Euscorpis*, from a thickening of the dorsal wall of the cerebro-optic invagination. The thickened portion consists at first of a mass of large spherical nuclei, which are distributed through the whole thickness of the retina. In the latest stage which I examined, in which there is already a considerable quantity of pigment, the nuclei are confined to the inner half of the retina, leaving the outer portion quite clear (fig. 18). The nuclei are, however, no longer all alike; but, while the majority of them retain their spherical form, a certain number, forming a band in the middle of the retina, have assumed an elongated form. These



latter are the nuclei of the retinal cells, and as from the distribution of the pigment special pigment-cells seem to be present, the large spherical nuclei probably belong to these latter. Absolute confirmation of this view by dissociation of the elements of the retina has unfortunately proved impossible, owing to their state of preservation.

### Conclusions.

The development of this form adds another to the numerous types of development in the Arachnida. It is, as is shown by its mode of nutrition, a highly specialised form. There is no doubt that the type of development represented by *Euscorpius* is the more primitive of the two. The chief arguments in favour of this view are the formation in *Scorpio fulvipes* of (1) a rudimentary amnion, and (2) the formation of yolk spheres in the earlier stages, and a mass of yolk round which the gut is formed.

Further, so far as can be made out from the description by Kowalewsky and Schulgin,<sup>1</sup> the development of *Androctonus* follows much the same lines as that of *Euscorpius*. It is curious that *Euscorpius* should resemble the very distantly related *Androctonus* so closely, while differing so markedly from a comparatively near relation like *Scorpio*; and further study of the mode of development in other forms would, probably, throw an interesting light on the value of the present system of classification.

The mode of nutrition explains many of the peculiar points in the growth of the embryo, everything being sacrificed to the rapid development of the organs—chelicerae, stomodæum, and gut—necessary for nutrition, and the other appendages, together with the mesoblast and nervous system, being formed at leisure after nutrition is provided for.

I have, unfortunately, not been able to find the remarkable sense organs described by Patten.<sup>2</sup>

<sup>1</sup> 'Biol. Centrbl.,' vol. vi, p. 525.

<sup>2</sup> 'Quart. Journ. Micr. Sci.,' vol. xxxi.

## EXPLANATION OF PLATE XL,

Illustrating Mr. Malcolm Laurie's paper on "Some Points in the Development of *Scorpio fulvipes*."

*Abbreviations.*

*am.* Amnion. *ap.* Appendix of diverticulum. *A. S.* Air-space. *Bl. S.* Blood-sinus. *c.* Collar of diverticulum. *co.* Cord of cells on which embryo feeds. *ep.* Epiblast. *est.* Epistome. *ht.* Heart. *hy.* Hypoblast. *i. l.* Inner layer of ovarian tube. *m.* Muscles of stomodæum. *mes.* Mesoblast. *n. c.* Nerve-cord. *o.* Central eyes. *o'.* Lateral eyes. *o. l.* Outer layer of ovarian tube. *o. d.* Ovarian tube. *o. n.* Optic nerve. *ov.* Ovum. *r.* Nuclei of retinal cells. *s.* Stalk of diverticulum. *st.* Stomodæum. *stg.* Stigma. *v.* Vitreous layer. *yk.* Yolk. The appendages are numbered I, II, III, &c. For the sake of simplicity the diverticulum round the embryo has not been figured, except in Fig. 14.

FIG. 1.—Part of ovary of *Scorpio fulvipes*, to show the diverticula in which the eggs are formed.  $\times \frac{80}{1}$ .

FIG. 2.—Longitudinal section of a diverticulum and ovum at an early stage.  $\times \frac{80}{1}$ .

FIG. 3.—Transverse section of the lower part of a diverticulum.  $\times \frac{118}{1}$ .

FIG. 4.—Transverse section of the swollen "collar" of a diverticulum.  $\times \frac{78}{1}$ .

FIG. 5.—Transverse section of the solid coiled appendix in which the diverticulum ends.  $\times \frac{78}{1}$ .

FIG. 6.—Transverse section of a fully segmented egg.  $\times \frac{250}{1}$ .

FIG. 7.—Transverse section of front end of a very young embryo, showing stomodæum, amnion, &c.  $\times \frac{250}{1}$ .

FIG. 8.—Longitudinal section of an embryo slightly older than Fig. 7.  $\times \frac{145}{1}$ .

FIG. 9.—Transverse section. Body of embryo considerably older than Fig. 8.  $\times \frac{160}{1}$ .

FIG. 10.—Transverse section through head of same embryo, showing stomodæum, chelicerae, &c.  $\times \frac{160}{1}$ .

FIG. 11.—View of the dorsal surface of an embryo, older than Fig. 9.  $\times \frac{80}{1}$ .

FIG. 12.—Transverse section of body of same embryo.  $\times \frac{60}{1}$ .

FIG. 13.—Transverse section of body of an older embryo.  $\times \frac{60}{1}$ .

FIG. 14.—Transverse section through head of same embryo, showing mode of nutrition.  $\times \frac{80}{1}$ .

FIG. 15.—View of ventral surface of advanced embryo. The iii to vi appendages have been removed on the left side.  $\times \frac{8}{1}$ .

FIG. 16.—View of mouth parts of same embryo, from the front.  $\times \frac{8}{1}$ .

FIG. 17.—View of mouth and surrounding appendages of same embryo. The left chelicera has been unfolded to show its structure, and the left chela has been removed.  $\times \frac{13}{1}$ .

FIG. 18.—Section through retina of central eye.  $\times \frac{300}{1}$ .





## Abstract of Maupas's Researches on Multiplication and Fertilisation in Ciliate Infusorians.

By

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THE interest so widely felt in all questions of heredity and reproduction removes all need for excuse in presenting an abstract of two of the most important contributions to this question.<sup>1</sup> I drew this up originally for my own guidance,<sup>2</sup> and have now enlarged it with a few modifications in what I may term "notation," to make the nuclear relations more easy to master.

The methods used are given at length. Cultures are made from single progenitors in a drop of water placed on a slide and covered, with bristles, &c., placed under the thin glass to prevent pressure, the total amount of water being about 10 cgms. These slides are kept in moist chambers, and the slight loss of water from evaporation is made up as needed with distilled water. The Infusoria under these circumstances live and thrive, assembling in a circle just within the cover, and not moving about much when well supplied with food, so that counting them is no difficult task. The Ciliata may be divided into herbivorous and carnivorous. The former are fed by

<sup>1</sup> "Sur la multiplication des Infusoires Ciliés," in 'Archives de Zoologie Expérimentale,' sér. 2, vol. vi, pp. 165—273, t. ix—xii; "Le Rajeunissement Karyogamique chez les Ciliés," op. cit., vol. vii, pp. 149—517, t. ix—xxiii.

<sup>2</sup> In the autumn of 1889; the abstract in its present form was completed in November, 1890.

cautiously supplying very dilute boiled paste to the colony. The latter require to be supplied with smaller Infusoria, for which purpose *Cryptochilus nigrescens* is raised in hay-decoction; the drop of the liquid with this form is always examined under the microscope to ascertain its freedom from other forms, and only after this control drawn off with the pipette to be given as food. In the research on fission observations were made daily, and entered with the temperature in the journal. Twenty species were examined, and their rate of bipartition carefully studied. For any given species this rate varies with (1) the temperature, (2) with the quantity, and (3) the quality of the food; while light or darkness is absolutely without influence.

It is found with each species that for temperature there is a minimum below which no bipartition takes place, an optimum at which it proceeds most rapidly, and a maximum beyond which, again, there is no occurrence of reproduction. Exhaustion of the food-supply determines encystment if the culture is of close relations; conjugation when the individuals are of mixed origin, and their age is suitable. This is a discovery on which the second research was largely based, as Maupas could now induce conjugation at will.

The greatest frequency of bipartition at temperatures of  $15^{\circ}$ — $18^{\circ}$  was observed in *Glaucoma scintillans*, namely, five times in the twenty-four hours, and the least in *Spirostomum teres*, viz. once in forty-eight hours, or one tenth the rate of the former species.

Maupas started the cultures with an individual that had just separated from its partner in conjugation, and which he terms an "exconjugate." In this way it has been possible to ascertain whether the rate of bipartition increases or diminishes as we recede from the ancestor; and he finds that neither change takes place at first, but that the rate is fairly constant for all the individuals of a single cycle for a long period, after which it diminishes: there are, however, fairly marked differences between the descendants of different ancestors of the same species.

Considering Weismann's assertion, that Protozoa are immortal, it is of extreme interest to note that the later individuals of a cycle differ from the preceding ones in a way that fully justifies Maupas's use of the term "senescence." The later individuals are reduced in size—sometimes to one fourth of their normal length; the buccal wreath is more and more reduced as the stock ages; the nuclear apparatus undergoes degeneration in various ways, rendering fertile conjugation impossible. In this stage, however, it is remarkable to find that sometimes a sexual hyperæsthesia is observed, inducing conjugation between close relations, though this is of course sterile, and accelerates the death and disappearance of the stock. This ultimate death by senescence took place in *Stylonychia pustulata* in 316 generations from the exconjugate progenitor; while in a stock of *Leucophrys patula*, of which the progenitor was possibly not an exconjugate, it occurred only after 660 generations.

In a section of the second paper these results are completed by the proof that for many species, at least, the cycle shows from its origin in conjugation to senescence first of all a stage of "immaturity," in which conjugation cannot be induced; then, after a certain number of bipartitions, "puberty" is reached, and during the period of "eugamy" that ensues fertile conjugation can be induced at any time by suitable treatment: this second stage merges into the last—of "senescence." Puberty is reached in *Stylonychia pustulata* at the 130th bipartition, in *Onychodromus grandis* at the 140th, and in *Leucophrys patula* only at the 300th; and senescence commences in the first about the 170th (though some senile individuals showed as early as the 100th), in the second at the 230th, and in the third at the 450th. Since bipartition proceeds in some cases in senile individuals that have lost their micronucleus, it is obvious that this organ plays no necessary part in the vegetative reproduction of the Ciliata.

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In the second paper we come to the study of conjugation, and the first full account of it yet given, illustrated by exquisite figures as well as by numerous diagrams, some of which we shall reproduce in a modified form. The materials were obtained by the methods described above, the starvation of mixed colonies of a species at a suitable stage always bringing about conjugation. In these experiments it was found that the method of starvation and mixing colonies of different stocks failed if tried too soon after conjugation, and this was the starting-point of the discovery of the puberty of the cycle mentioned above.

In some cases (e. g. *Leucophrys patula*) the first result of starvation is to induce the production of dwarf forms by repeated fission, and it is only these dwarf forms that conjugate; if, however, a food-supply be introduced at a sufficiently early stage, the gametes quit company, and resume the ordinary process of growth and bipartition. The formation of similar dwarf forms occurs only in certain members of the stock of Vorticellines, and eventuates in the production of the microgametes or "males," which swim to and conjugate with the undivided attached megagametes.

By careful observation the time for each stage of the process was determined; and by killing and fixing couples at successive intervals, preparations of every stage were easily obtained for research. The fixative was a 1 per cent. solution of corrosive sublimate, and the animals were stained on the slide with picro-carmin or a solution of methyl green in 2 per cent. acetic acid, and then cleared in situ with glycerine or balsam. The best homogeneous immersion objectives are absolutely needed for a complete study of these processes.

I may recall the fact that the nuclear apparatus of Ciliata is double, comprising the large "meganucleus" (macronucleus, endoplast, nucleus of authors), and the "micronucleus" (endoplastule, paranucleus, or nucleolus), which latter is multiple in some species. In bipartition the meganucleus divides by mere constriction, while the micronucleus shows all the stages of karyokinesis save the disappearance of its membrane. In



conjugation a pair of Ciliata approach and adhere, fusing more or less completely by their oral surfaces, and then separate after a time: it has been long known that in this process the nuclear apparatus undergoes a complete reconstruction. What the nature of this reconstruction is, Maupas has now revealed.

The process of conjugation is divided by the author into the eight following stages. In A the micronucleus enlarges greatly. In B, C, D it undergoes successive bipartitions. Two sister nuclei of the third bipartition (stage D) are specialised as "pronuclei:" the one of these migrates into the other gamete to fuse with the stationary pronucleus there; and this process constitutes stage E. The "copulation-nucleus" so formed undergoes two fresh bipartitions in stages F and G. In stage H the nuclear apparatus is reconstituted and the first bipartition takes place.

In the five stages B, C, D, F, G, involving nuclear division, we can distinguish the following substages or phases: (1) Spirema, the nucleus shows a reticulation; (2) aster, the chromatin forms distinct thickish rods, extending the greater part of the fusiform nucleus, its poles being occupied by fine achromatic filaments; (3) equatorial plate; (4) diaster, the chromatin rods are in two groups united by achromatin filaments; (5) dispirema, the two chromatin groups are more distant, and contorted, and united by achromatin filaments which are strangulated in the middle, or contained in a dilated connective tube (boyau), which is soon absorbed into the ambient cytoplasm. Thus  $G_2$  would indicate the aster stage of the second bipartition of the copulation-nucleus.

In stage A we may also distinguish four substages: (1) the micronucleus unchanged; (2) swelling without much change of form; (3) further swelling with changes of form characteristic of the species; (4) the micronucleus condenses and shrinks by way of preparation for its first bipartition in stage B.

Stage E is also divided into phases:  $E_1$ , the male pronucleus approaches the point of exchange;  $E_2$ , it passes into the fellow-

gamete;  $E_3$ , it reaches and touches the female pronucleus;  $E_4$ , the male and female pronuclei fuse completely to form the copulation-nucleus.

Stage H is divided into the following phases:  $H_1$ , the offspring of the copulation-nucleus are all similar;  $H_2$ , they are differentiated into enlarging meganuclei and small micronuclei;  $H_3$ , the meganuclei still enlarge, but refuse nearly or altogether to take up stains;  $H_4$ , completion of the growth of the meganuclei, which now stain well; the exconjugate is now ready to undergo bipartition.

The separation of the exconjugates usually takes place in stages F and H. I have tabulated the cases given by Maupas, and find that in one species they separate in E, four in F, three in G, eight in H; while in one species, *Spirostomum teres*, they may separate in any stage from  $F_3$  to  $H_4$ .

For the sake of additional clearness, I have used the following notation,  $\mu$  or  $\nu$  = the micronucleus of a gamete; Z, the copulation-nucleus formed by the fusion of the two pronuclei. M, the mega-, and  $m$ , the micro-nuclei of the exconjugate. To denote the daughter nuclei after any given number of mitoses I use this same number as an index to the letter of the original nucleus; thus  $Z^1$  (abbreviated for  $Z \div 2^1$ ) represents one of the two nuclei formed by the first mitosis of the copulation-nucleus;  $\mu^3$  is one of the nuclei resulting from the third mitosis of the original micronucleus of a gamete.

We may now describe fully the process in one of the simplest forms, *Colpidium Colpoda*, which has a single micronucleus. This enlarges and divides twice in stages B and C; of the four nuclei so formed, three abort and are finally absorbed by the cytoplasm, as "corpuscules de rebut;" the fourth undergoes a new bipartition (stage D) to form the two pronuclei of the formula  $\mu^3$ . The point that determines which of the four nuclei,  $\mu^2$ , should be preserved for bipartition seems to be the accident of position; it is always the one at the frontal end of the animal.

So, too, position determines the respective fates of the two pronuclei; that which is next the point of union of the

two gametes is the male or migratory pronucleus, the other the female or stationary pronucleus. In stage E each male pronucleus crosses over the other at the point of contact of the gametes, and fuses with the female pronucleus of the other gamete: the copulation in this species takes place in the resting stage of the pronuclei. Each conjugate has now a "copulation-nucleus," which we letter Z.

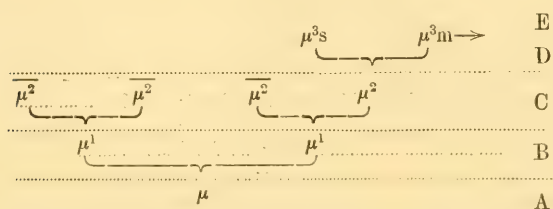


FIG. 1.—Schema of nuclear processes in a conjugating Ciliate, from the first division of the micronucleus ( $\mu$ ) to the formation of the conjugating pronuclei ( $\mu^3s$ ,  $\mu^3m$ ). The arrow indicates the path of the migratory pronucleus; a bar is placed above the rejection nuclei. The dotted lines separate the successive stages, marked by capital letters at the right hand.

The first mitosis, stage F, takes place in the front of the body, but the nuclei  $Z^1$  pass to the hinder half to divide again in stage G. By the elongation of the connective tubes in the dispirema stage,  $G_5$ , two of the nuclei  $Z^2$  pass into the anterior end and become meganuclei; and their respective sisters pass to the hinder end and become the micronuclei of the offspring of the first bipartition.

Separation takes places in the stage  $H_3$ ; and young exconjugates seem to have neither mouth, gullet, nor frontal prominence: from three to eight days elapsing before the mouth forms afresh and feeding recommences; and from eighteen to twenty-four hours more until the first bipartition. Before this takes place the micronuclei move up and place themselves beside the meganuclei; one set goes to the fore, the other to the hinder half of the body; while a second contractile vacuole appears in the front half. Transverse fission then takes place.

"It is obvious," says Maupas, "that this division, unprovoked by any nuclear division, must be due to the forces of the protoplasm, the nuclear elements only playing a passive part."

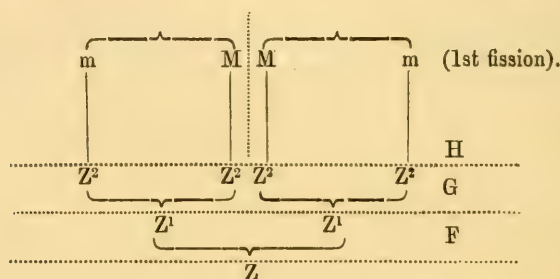


FIG. 2.—Reconstitution of nuclear apparatus from conjugation-nucleus in an exconjugate *Colpidium Colpoda*, and reversion by a single fission to the normal type with single mega- and micro-nucleus (M, m). The dotted vertical line indicates the plane of fission.

We may express this by equations thus :

$$\begin{aligned}
 Z &= 4 Z^2 && \text{(Stages F, G).} \\
 4 Z^2 &= && \\
 &= 2 M + 2 m && \text{(Stage H).} \\
 &= \{M + m\} + \{M + m\} && \text{(1st fission).}
 \end{aligned}$$

The original meganucleus is passive throughout, and retains its granular structure till  $H_2$ . At  $H_3$  it becomes homogeneous, and still stains well; while in  $H_4$  it passes to the hinder end of the body, becomes smaller and irregular, loses its staining power, and finally disappears completely. The above diagrams (Figs. 1, 2) are modified from Maupas.

We may sum up matters thus:—In each gamete the micro-nucleus undergoes three bipartitions; two sister nuclei of the third bipartition are differentiated into stationary or female pronucleus, and migratory or male pronucleus respectively. By fusion of a male and female pronucleus a copulation-nucleus is produced; by two successive mitoses are formed four nuclei of the formula  $Z^2$ ; the sister nuclei of either pair are differentiated in their development to form the one a mega-



the other a micro-nucleus; and on fission each set so formed constitutes the nuclear apparatus of the first offspring of the exconjugate. During these processes the original meganuclei of the gametes have undergone disorganisation and absorption.

We may express the above still more briefly thus:—Two Ciliata of the same species approach by their oral surfaces; the original micronuclei by repeated bipartition form a pair of pronuclei; one pronucleus in either gamete migrates into the other to fuse with the stationary pronucleus therein; then a new nuclear apparatus is constituted by the bipartitions of the copulation-nucleus; the gametes now separate to found each a new life-cycle of the species.

Several important variations occur on the schema given above; thus, in *Coleps hirtus*, &c., with a single micro-nucleus, stage G is doubled ( $\frac{G}{1} \frac{G}{2}$ ), so that we have eight nuclei of the form  $Z^3$ , instead of four of the form  $Z^3$ ; two of these become micronuclei, as in *Colpidium*; and it seems probable that the other six are transformed into meganuclei, which, by interfusion, have their number reduced to 2.

As a more complicated variation I may cite *Paramœcium caudatum*, where the stages are identical with *Colpidium* as far as E, and stage G is doubled as in *Coleps*.

We may represent the process of reconstruction in (a single exconjugate) *Paramœcium caudatum* by the following equations, using the symbol for multiplication by zero ( $\times 0$ ) to designate the elimination of certain of the nuclei descended from the conjugation-nucleus:

$$\begin{aligned}
 (a) \quad Z &= 8Z^3 & (\text{Stages F, } \frac{G}{1}, \frac{G}{2}). \\
 (b) \quad 8Z^3 &= 4M + m + 3Z^3 \times 0 & (\text{Stage } \bar{H}). \\
 (c) \quad 4M + m &= 4M + 2m^1 = \{2M + m^1\} + \{2M + m^1\} & (\text{1st fission}). \\
 (d) \quad 2M + m^1 &= 2M + 2m^2 = \{M + m^2\} + \{M + m^2\} & (\text{2nd fission}).
 \end{aligned}$$

Here one of each of the four pairs of nuclei  $Z^3$  becomes a meganucleus, their sister (micro-) nuclei all aborting save one. This divides during the first bipartition, of which the offspring

have hence two meganuclei; and it is only at the second bipartition that the nuclear apparatus is reduced to one mega- and one micro-nucleus as characteristic of the species. The primitive meganucleus of the gametes undergoes fragmentation into as many as sixty parts, which persist in the ex-conjugates and their offspring for some time; they usually undergo absorption, or are expelled as fæces; but if the culture is starved, some may fuse with the new meganucleus.

*Paramœcium aurelia*, with which *P. caudatum* is usually confounded, has two micronuclei, so that at stage C eight nuclei of the formula  $\mu^2$  are present; only one of these undergoes the next bipartition to form the pronuclei, all the others being "corpuscules de rebut." Stages F and G are as in *Colpidium*; but at the first bipartition the micronuclei divide, one daughter nucleus of each going to either of the offspring. The following equations represent Stage H and the first fissions of *Paramœcium aurelia*:

$$\begin{aligned} 4Z^2 &= 2m + 2M && \text{(Stage H).} \\ 2m + 2M &= 4m^1 + 2M. \\ &= \{2m^1 + M\} + \{2m^1 + M\} \text{ (1st fission).} \end{aligned}$$

Still more complicated is the process in *Vorticellines* where the gametes are of unequal size, the smaller ones being formed by vertical fissions of an individual into four or eight, and swimming freely. One of these microgametes conjugates with a megagamete and fuses completely with it, so as to form a single zygote—a process first fully described by Stein. Either gamete here only forms a single functional pronucleus, their sister pronuclei aborting. As the two pronuclei advance to meet they might, perhaps, be regarded both as equivalent to the male pronuclei of other species; but it is impossible to regard the pronuclei as really differentiated into distinct sexes. Stage G is double, so that eight nuclei are formed. Of these, seven evolve into meganuclei; one into the micro-nucleus of the zygote. Successive fissions of the zygote take

place, in which the meganuclei are distributed between the offspring, while the micronucleus undergoes bipartition: the normal state of the species is reached in one offspring of the second, and in six of the third fission.

The processes of nuclear reconstitution in Vorticellines, with the fissions necessary to bring back the type form, possessing a single mega- and micro-nucleus, are represented in the following equations. In each case a vinculum — is put above the normal form.

$$\begin{aligned}
 8Z^3 &= 7M + m && \text{(Stage H).} \\
 7M + m &= 7M + 2m^1 = \{3M + m^1\} + \{4M + m^1\} && \text{(1st fission).} \\
 3M + m^1 &= 3M + 2m^2 = \{\overline{M + m^2}\} + \{2M + m^2\} && \text{(2nd fission).} \\
 4M + m^1 &= 4M + 2m^2 = \{2M + m^2\} + \{2M + m^2\} && \text{,, ,,} \\
 2M + m^2 &= 2M + 2m^3 = \{\overline{M + m^3}\} + \{\overline{M + m^3}\} && \text{(3rd fission).}
 \end{aligned}$$

The relations are further complicated by the reduplication of the micronucleus of the microgamete in the beginning of stage A; both these micronuclei undergo the bipartitions of stages B and C; but only one of the eight nuclei ( $2\nu^2$ ) so formed divides in stage D, which is thus identical with that of the megagamete. A similar reduplication of the micronucleus occurs in both the isogamous gametes of Euplotes.

The fusion of the two cytoplasms takes place at the end of stage E. The old meganucleus undergoes fragmentation, and its pieces may be all absorbed, or some may finally fuse with the new meganuclei.

I have cited a number of distinct cases from Maupas to show that, with wide differences of detail, the phenomena are everywhere essentially the same in the Holotricha, Heterotricha, Hypotricha, and Peritricha: his observations on the Suctoria or Acinetina show that the law may be extended to this group—a sure proof, if one were needed, of its true affinities.

A series of elaborate discussions follows the systematic review of the phenomena of conjugation in the Ciliata. The double nature of the nuclear apparatus in this group is explained by a

physiological division of labour. The MEGANUCLEUS presides over the nutrition and growth of the individual, and holds the power of repairing injuries ; it divides by simple constriction. The MICRONUCLEUS presides over the preservation of the species, and is the seat of that power of rejuvenescence which allows continued reproduction, and the substratum of inherited qualities ; it always divides by mitosis.

In the mitosis of the micronucleus the connecting tube of the two daughter nuclei always seems to be lost by absorption into the cytoplasm, and as this contains the greater part of the nucleo-hyaloplasm, we may regard it as an additional proof that the chromatin is the essential element of the nucleus, in which reside its special functions, those of transmitting inherited properties ; while the hyaloplasm has a purely mechanical function. The greater or lesser compensation for loss of substance in mitosis is effected by osmosis only, as the nuclear wall always persists.

It is shown that fertilisation is essentially a nuclear phenomenon : the union of the chromatic elements of two nuclei of different origin so as to constitute a new " nucleus of rejuvenescence." As to its physiological purport, it is very obvious that the common view is wrong, and that fertilisation is not necessarily linked with multiplication. In Ciliata fertilisation interrupts the ordinary rapid multiplication of the race for hours and days together. Thus *Onychodromus grandis* is six days in conjugation ; in which time by fission each gamete could have produced in the thirteen bipartitions some 8000 offspring ; while in Vorticellines the one of the gametes is practically suppressed and its power of fission annihilated.

Rolph's theory of isophagy is only mentioned to be waived as not corresponding with the facts.

Let us now examine Weismann's view : that sexual or amphigonic reproduction has the chief and sole function of mixing inherited tendencies, and of creating individual differences, through which selection forms new species. This view excludes fecundation from all share in the sustenance and preservation of the original species, on the ground that living matter pos-



essed as a primordial attribute an unlimited power of growth and reproduction, as inherent as energy is in matter, and quite independent of the actual mode of reproduction, sexual or asexual.

This latter part of Weismann's theory is completely overturned, so far as the Ciliata are concerned, by Maupas's proof of the existence of senescence and death as the normal termination of every cycle, when the possibility of normal conjugation is excluded. Thus the hypothesis of the essential immortality of the Protozoa has lost its foundation in fact.<sup>1</sup>

Maupas's own conclusion as to the real explanation is, that fertilisation determines rejuvenescence, a view enunciated by Bütschli and Engelmann, and adopted by Hensen and E. van Beneden.

Fertilisation is, in its essence, independent of sexual differentiation. This is clearly shown by the above studies on the Ciliata, where both gametes and pronuclei are clearly identical. Henceforward we can only look on sex as a secondary adaptation to facilitate the act of fecundation.

Besides rejuvenescence, fecundation does determine the transmission of inherited qualities; and we must admit this part of Weismann's view: that it plays an important part in variability.

What is the essence of this act of fecundation considered as a general phenomenon of organic life? Maupas answers this question in the following theses:

1. Morphologically it is essentially a nuclear phenomenon.
2. The nuclei retain their existence throughout; Haeckel was mistaken in assuming their disappearance in a "moneran stage."
3. A preliminary reduction takes place in the nuclear substance, which by successive normal mitoses is reduced by three fourths; the parts so eliminated disappear by absorption as "noyaux de rebut," or else are expelled as polar bodies.

<sup>1</sup> My own standpoint is somewhat different, for I admit the existence of primitive forms, like the *Monadina* of Cienkowski, that are completely agamous and "immortal."

The reduction so effected is purely quantitative, and appears to involve male as well as female cells. (The grounds on which this thesis is founded are referred to below.)

4. "Noyaux de rebut" and fertilisation-nuclei are absolutely equivalent; their respective fates are determined by the accident of position.

5. By the last mitosis both "noyaux de rebut" and fertilisation nuclei cease to be true nuclei, capable of indefinite normal evolution, and are simple pronuclei, only possessing restricted powers of development.

6. The peculiar characters of the pronuclei are due then to the preliminary double mitosis; they can only accomplish their functions by union (copulation) with a nucleus from a different germinative cell.

7. Normal copulation can take place between two pronuclei, and two only.<sup>1</sup>

8. The copulating nuclei, though of distinct origin, are equivalent to one another, and either plays the same part to the other. In the nuclear union, the supreme act of fecundation, there is neither male nor female; the sexual modifications, which are so obvious to our eyes, are mere accessory adaptations to facilitate and ensure the approximation of the pronuclei, which themselves are of no sex at all. Fecundation, reduced to its lowest terms, is distinct from and independent of sexuality.

9. The high evolution of these accessory sexual adaptations is only a proof of the extreme physiological importance of the nuclear copulation in fecundation.

10. The chromatin of the nuclei represents their permanent personality; the other nuclear structures are subject to continual changes, destructive and recuperative, and only play an accessory part.

11. The act of fecundation is completed by the union of the chromatic elements of the two pronuclei into one single nucleus, and this union may be effected at different stages of nuclear evolution.

<sup>1</sup> Maupas seems ignorant of the many cases of "multiple isogamy" in Protohytes: see "Some Problems of Reproduction," in this Journal.

12. Probably the chromatic elements of the two pronuclei retain their individuality in the copulation nucleus, while only the nuclear accessories, their hyaloplasmas and juices, undergo complete fusion. If this be so, fecundation might be stated as the approximation of chromatic elements of two distinct origins, and their incorporation into a single nucleus.

The above statements, No. 3 especially, require fuller explanation. In the first place, while two mitoses are usually required for the formation of the female pronucleus in Animals, three, at least, are always requisite in the Ciliata; a point which Maupas dismisses as "a special adaptation of the organism of these Protozoa." His attempt to carry on this rule to the vegetable world is very forced: it can, indeed, only be adopted for the Archegoniata, where the successive formation of neck- and belly-canal-cells compares very fairly with the formation of polar globules in Animal ova; the first of which is known to divide again in some Animals, like the neck-canal-cell of many Archegoniates. But the identification with the processes in the embryo-sac of Flowering Plants is only carried out by the suggestion that all the authors who have described the evolution of this structure are wrong in their description! Maupas admits that there is difficulty in finding similar mitoses, and anything corresponding universally to "*corpuscules de rebut*" in the evolution of spermatozoa, though some cases present marked homologies. He also interprets the "*vegetative nuclei*" of the pollen as *corpuscules de rebut*, which may be physiologically correct, but finds, at least in part, another, morphological, explanation. He points out that all authors are agreed in regarding the production of polar bodies as involving the expulsion and elimination of a quantity of nuclear substance useless for the normal evolution of the germ cells; but that Weismann's view as to the significance of this elimination is disproved by the absolute identity of polar and germ-nuclei in Metazoa; and of the sister offspring of the micronuclei in the Infusorian gametes. For the present Maupas abstains in the chapter on a "General Theory of

Fertilisation" from giving any explanation of his own; but in the preceding one he suggests that "we may always suppose that a reduction in the quantity of material elements may act with effect on molecular forces, attenuating their strength and giving more delicacy to their play." Now, what we know of molecular forces would be rather in favour of the view that reduction of the masses would give more energy and violence to the action of the nuclei. He is clearly right in rejecting all explanations of "polar bodies" which are inapplicable to the three preliminary mitoses of the micronucleus of the infusorian gamete.<sup>1</sup> Probably the phenomena are essentially morphological, a question which will be discussed in my paper on "Some Problems of Reproduction."

In the above abstract many interesting points have been left untouched; much has been treated far too summarily. But I trust that I have given an idea of the value and wealth of the papers, and brought out their two cardinal points: the limits of reproduction by continuous fission alone; and the nature and results of conjugation.

<sup>1</sup> A singular omission from this discussion is that Maupas nowhere brings in his discovery that certain brood-nuclei of the rejuvenated conjugation-nucleus itself abort as "noyaux de rebut," instead of becoming mega- or micro-nuclei.



On the Occurrence of Pseudopodia in the Diatomaceous Genera, *Melosira* and *Cyclotella*.

By

**J. G. Grenfell, B.A., F.G.S., F.R.M.S.**

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With Plate XLI.

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THE diatoms on which these pseudopodia have been found do not belong, as might have been expected, to the motile, but to the non-motile forms, to the genera *Melosira* and *Cyclotella*. The *Cyclotella* is certainly *C. Kützingiana*. The *Melosiras* belong to one or two small species which have not yet been satisfactorily determined.

They were first met with in April, in the large pond in the gardens of the Royal Botanical Society of London, in the Regent's Park. This gathering consisted almost entirely of small isolated frustules of *Melosira*, with a few *Cyclotellas* and some *Archerina Boltoni*.<sup>1</sup> Later on filaments of frustules of *Melosira* became commoner, while *Archerina* increased enormously in number. Finally *Cyclotellas* replaced the *Melosiras*, and the *Archerinas* vanished.

I next met with pseudopodia on *Cyclotellas* at Stanstead, in Hertfordshire, whither I was directed by a friend.

Later I went to stay at Heytesbury, in Wiltshire, where I found the river Wiley and the brooks full of a *Melosira* in small isolated frustules, with long, delicate pseudopodia. Recently I have found a good set of *Cyclotellas* with pseudopodia in Kew Gardens, and others at Eastbourne. I infer that these

<sup>1</sup> See Professor Lankester's description of this organism, 'Quart. Journ. Micro. Sci.,' vol. xxiv, 1884.

small isolated *Melosiras* and *Cyclotella Kützingiana* have these pseudopodia normally. At Stanstead, Heytesbury, Kew, and Eastbourne there was no trace of *Archerina*. The reason for mentioning this is that it was suggested that the external protoplasm of *Archerina* migrated on to the diatoms.

The pseudopodia of the first gathering and of the Stanstead ones were easily seen for a part of their length with a  $\frac{1}{6}$  object-glass, magnifying nearly 400 diameters; but in the case of the Heytesbury *Melosiras* and most of the *Cyclotellas* from the Botanical Gardens and Kew they are generally invisible, even when specially looked for. I think this helps to explain why they have not been found before.

To study the whole length of the pseudopodia I found it a good plan to dry the material on a cover-glass, which could then either be mounted dry or stained or roasted. All the figures on Pl. XLI are from specimens thus treated. Quite lately I have stained and mounted some of the Kew gathering without drying. The results I hope to give in a future paper. So far they have simply confirmed the main conclusions at which I had already arrived.

The principal points to notice in the structure of the pseudopodia are these: they are fairly stiff, and are non-retractile to ordinary observation.

The length varies in *Cyclotella* from two and a half to six times the width of the valves. In the Heytesbury *Melosiras* this reaches fully nine times the width. They are very permanent; a slide prepared in April by simply sealing the diatoms in the water in which they were found showed the pseudopodia apparently unchanged after five months. The dried slides show that the great majority of the pseudopodia are arranged fairly symmetrically round the margin of the valves. This is best seen in side view (figs. 6 and 7).

In slides of the Kew gathering prepared in the wet way a great many *Cyclotellas* show a series of small tooth-like projections of protoplasm round the margins of the two valves, arranged as regularly as the teeth of a circular saw. These projections are the thicker bases of the delicate pseudopodia. On

a typical specimen I counted about forty-six of these projections, while a roasted cover of the same gathering gave about forty-six as the number of radiating ribs on the valve of the *Cyclotella*. Hence there would appear to be a close connection between the number of the pseudopodia and the structure of the diatom—a point of very great importance. Sometimes pseudopodia spring from the surface of the cingulum as well as from the valves.

The pseudopodia are generally fairly straight; occasionally they branch at some distance from the valve. This was especially the case in the earliest gathering (figs. 1 and 2), where they branched repeatedly.

On dried cover-glasses it is common to find two or three pseudopodia springing from a short thickened base. In water unstained these bases are extremely hard to see.

The number of the pseudopodia is, on the whole, strikingly regular. On dried covers seventeen to twenty is the ordinary number seen round a valve in side view (fig. 6); sometimes twice that number (fig. 7).

The pseudopodia vary a good deal in thickness in different gatherings. On dried unstained cover-glasses they often show short portions of their length more opaque and solid-looking than the rest, sometimes getting a beaded look. In this they agree with the pseudopodia of *Archerina* (figs. 5, 6).

Rarely one comes across quite thick pseudopodia. Fig. 5 represents a striking form, with a few very stout ones. It is noteworthy here that these four spring from the coarser markings on the valve. Occasionally one of the pseudopodia is thickened in the centre, as in fig. 6. Lastly, the pseudopodia of two diatoms seem to be able to fuse into each other, and increase greatly in width, so as to form a broad band connecting the two diatoms. Whole chains are thus formed, though two frustules only are more common (figs. 2 and 8).

Next as to the use of these pseudopodia, and the question why other diatoms do not have them. The chief point to be remembered is that these little *Melosiras* and *Cyclotellas* occur mainly as isolated frustules, and are without the power of loco-

motion. Under these circumstances the pseudopodia serve three purposes: 1. Protection. 2. Means of attachment. 3. Floats.

1. Protection.—The pseudopodia act in the ordinary way as defensive spines. I have often seen large predatory Infusoria knocking these about, and absolutely unable to touch them. The ordinary isolated diatoms can creep into mud or débris out of harm's way. To these the stiff pseudopodia would be quite useless.

2. Means of Attachment.—At Heytesbury I found the diatoms in running water, especially amongst filamentous weeds. Here the use of the pseudopodia is quite obvious.

3. Floats.—In the Botanical Gardens they are all over the still waters, and no doubt the large extra surface given by these pseudopodia helps to keep them floating. The remarkable pelagic diatoms *Chætoceros* also have long processes, but of a different kind: that is to say much coarser, and obviously forming part of the siliceous skeleton.

The next question is the substance of which these pseudopodia are made. I think the facts point conclusively to that substance being protoplasm. They are destroyed by nitric acid, while those of *Chætoceros* are not. All the finer parts are at once destroyed by roasting at the lowest red heat. The thick connecting bands and the thickened bases of the pseudopodia will stand a low red heat occasionally, while they, too, are entirely destroyed by a strong heat.

They stain readily with Kleinenberg's hæmatoxylin. With Schultze's solution they give no cellulose reaction, nor with iodine and sulphuric acid. Boracic carmine, which does not stain cellulose, stains the bases of the pseudopodia strongly, and just the same colour as the cell-membrane—the fine part slightly. It is quite probable that some kind of cuticle is secreted by the protoplasm in contact with the water, and that this gives to the bases and connecting bands their resisting power at a low red heat. The bases and cell-membrane always behave in nearly the same way to stains. Two other proteid stains, said not to stain cellulose, were tried. Picro-nigrosine stained the diatom bodies well, the bases fairly, and also the



finer parts. Alcoholic safranin just stained the pseudopodia; but all alcoholic stains are apt to fail with them.

The evidence of the stains, therefore, joins with the other tests in pointing to the presence of protoplasm with or without a very fine cuticle of uncertain nature. The thick pseudopodia, with their variation in density (fig. 5), also point to a very fine cuticle with protoplasmic contents. There is no evidence in any of my slides of a layer of protoplasm normally present outside the diatom shell. The pseudopodia or their bases spring straight from the shell. Most probably there is a fine layer of protoplasm outside the shell, for Imhoff has shown that the internal protoplasm reaches the surface, but it is not thick enough to be visible in optical section.

In the Kew gathering I have met with two or three specimens surrounded by a thick layer of what looks like a gelatinous substance, not granular. The pseudopodia, however, are independent of this, and are clearly traced through it up to the margin of the shell of the diatom.

I have also seen granular or fluffy substance round *Cyclotella*, which might be protoplasm; but this is not at all common. I have seen it occasionally round other forms which have no pseudopodia.

Next we come to what has been said or suggested as to the meaning of the pseudopodia.

The first suggestion was that these were not diatoms at all, but the identification of a well-known species, *Cyclotella Kützingeriana*, with pseudopodia has settled that point. Next it was suggested they might be extensions of the gelatinous layer which Professor H. L. Smith has shown to surround many diatoms.

But there is no evidence here of any kind of normal gelatinous envelope, and the stiffness of the pseudopodia and the permanence of the bases tell against this theory, which is further negated by the fact already stated, that the pseudopodia penetrate the gelatinous layer when this is present.

A third hypothesis, based on the remarkable slides where the diatoms and *Archerina* are mixed in countless profusion, is

that the pseudopodia are a vegetable growth covering everything in the field. But here the Heytesbury set are conclusive: on a single slide you may have some hundreds of diatoms of various kinds; about 200 may be the small *Melosira*, nearly every one of which will have pseudopodia, while nothing else in the field has any. Besides, botanists will, I think, agree that there is nothing plant-like in these forms. Another suggestion was that they are like the filaments of *Polysiphonia*. But these latter never branch and are not granular, and could not form connecting bands.

Another class of suggestions was that these pseudopodia do not belong to the diatoms, but to an investing animal like *Vampyrella*, which devours *Gomphonema*. But if they belong to an investing animal, where is the animal which invests? *Vampyrella*, any way, is visible both when wandering in search of diatoms and when investing. Besides, *Vampyrella* projects its pseudopodia from any part of the diatom, while here they are mainly confined to a definite tract. And *Vampyrella* and other predatory animals do not have pseudopodia so regularly symmetrical, nor so constant in number. I have seen what appeared to be Leidy's *Biomyxa vagans*, which is something like *Vampyrella*, investing diatoms. And here also the pseudopodia had no relation whatever to the structure of the diatom, while the animal itself was clearly visible outside of the diatom. But, strongest proof of all, *Vampyrella* devours the diatom and kills it in a couple of hours; while these diatoms lived for five weeks in one bottle, quite healthy all the time, and dead ones were not found.

I do not think that any theory based on *Vampyrella* or vampyrelloid animals (as suggested at the British Association) will explain the facts. If the pseudopodia are foreign to the diatom, it would have to be a case of symbiosis between the diatoms and unknown invisible animals. I know of no similar case.

All the phenomena seem to me to point to these pseudopodia being filamentous extensions of the cell protoplasm, probably strengthened by cuticular deposit.

The fact that they do not move or quickly retract under stimulus would not necessarily distinguish them altogether from true pseudopodia ; at any rate, if the processes of *Archerina* are to be called by that name these must also, for it is impossible to separate them. It might, however, be an advantage to have some distinctive name for such stiff, slowly changing or unchanging pseudopodia.

It remains to treat briefly of the morphological resemblances between these diatoms and other forms. In doing this I wish simply to point out resemblances or differences, not to draw any large conclusions.

The first point that strikes one about these pseudopodia is their extreme unlikeness to any plant or part of a plant. Secondly, it is remarkable that when pseudopodia are discovered on diatoms these resemble a type which, so far as I know, is absolutely confined to the Heliozoa, forms with which the diatoms are already connected by the presence of a more or less siliceous test. All the Heliozoa are characterised by the relative stiffness of their pseudopodia ; but in *Archerina* this relative stiffness becomes practically absolute, as in these diatoms. I do not know of any described form except *Archerina* which has these non-retractile pseudopodia.

But the similarity in type of the pseudopodia is not confined to rigidity ; it extends to general form. As far as I know, every single detail of form in the diatoms can be matched amongst the Heliozoa with the exception of the repeated branching of those of *Melosira*. The resemblance of the diatom's processes to those of *Archerina* is still closer : nevertheless in 99 cases out of 100 it is quite easy to distinguish on a dry slide the pseudopodia of *Archerina* from those of the diatoms.

They are distinctly wider and taper regularly. But in the 100th case you get an *Archerina* colony the pseudopodia of which are absolutely undistinguishable from those of the diatoms. Their size and shape are the same as those of *Cyclotella*, and on a dry cover they show the thickened bases and the denser portions of the filament which I have described as characteristic of the diatoms.

If one bears in mind that the little *Melosiras* have very little silica, possibly none at all at times, and that both they and the *Archerinas* contain chlorophyll bodies, the resemblance becomes still more striking. It is also a striking fact that the connection of the diatoms by bands of protoplasm finds its counterpart amongst *Heliozoid* Protozoa, as in *Monobia confluens*. Another point of agreement is the fact that *Archerina* at times has very few but very large pseudopodia, just like the *Cyclotella*.

A fact about *Archerina* as yet unpublished is worth notice. In staining with Schultze's fluid and with iodine and sulphuric acid I obtained the clearest evidence that the cuticle of the chlorophyll bodies is made of cellulose. In this it is more plant-like than the diatoms themselves, which do not give the cellulose reaction. Cellulose has been found in a number of animals, but still it is interesting that while the pseudopodia draw the diatoms nearer to the animals, this cellulose draws *Archerina* nearer to the plants. This fact is likely to be used as an argument in favour of *Archerina* being a case of symbiosis, and such a view may be extended to these diatoms. I will not discuss that view now. I do not think it is conclusive in the case of *Archerina*, much less in the case of the diatoms, where all the facts seem to point to the pseudopodia being integral portions of the diatoms.

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## EXPLANATION OF PLATE XLI,

Illustrating Mr. J. G. Grenfell's note "On the Occurrence of Pseudopodia in the Diatomaceous Genera, *Melosira* and *Cyclotella*."

FIG. 1.—*Melosira* of the first gathering in the Botanical Gardens, much branched. The second frustule shrivelled in drying. Stained.

FIG. 2.—*Melosira* of the same gathering. Stained.

FIG. 3.—*Melosira* filament from Botanical Gardens. Stained.

FIG. 4.—From Heytesbury, in Wiltshire. Unstained.

FIG. 5.—*Cyclotella* from Botanical Gardens. Four thick granular pseudopodia. Unstained.

FIG. 6.—*Cyclotella* *Kützingiana* from Botanical Gardens. Unstained.

FIG. 7.—Ditto, with more pseudopodia.

FIG. 8.—Two forms from Botanical Gardens, roasted at low red heat, showing projections and connecting band.



## REVIEW.

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**A** Monograph of the Victorian Sponges, by ARTHUR DENDY, D.Sc., F.L.S., Fellow of Queen's College, and Demonstrator and Assistant Lecturer in Biology in the University of Melbourne. PART I.—The Organisation and Classification of the Calcareous Homocœla, with Descriptions of the Victorian Species (with plates i—xi). Melbourne, July, 1891.

UNDER the above title a most interesting paper on Sponges has appeared, giving, it is not too much to say, the first attempt at an accurate description of the histology of the lower Calcareous since Metschnikoff's paper in 1879. I have been working at the same group myself for about half of the last five years. As it is likely to be still some months before my results appear, I think that it may facilitate discussion, while noticing some of the principal features in Mr. Dendy's paper, to indicate at the same time the extent to which my work has led me to similar or different conclusions. And I may commence generally by saying that in the plates of this paper I have had for the first time the pleasure of seeing drawings which represent accurately the structures which have come under my notice.

The introductory criticism on Haeckel (p. 3) appears somewhat to underrate the power of variation in calcareous sponges, particularly their plasticity to environment. Thus *Sycandra raphanus* has formed a special variety in the Naples Aquarium,

unknown in the bay and hitherto undescribed. It outwardly resembles *S. capillosa*. Vosmaer's views on *Leucandra aspera* ('Mitt. Zool. Stat. Neapel,' vols. iii and iv) may require some modification, but its variation is certainly enormous. It is true that the *Homocœla* of Naples seem fairly constant in outward form and canal-system (*sensu* Dendy), but evidence points to their being identical with species of very different aspect in other localities. I disagree with Mr. Dendy that "there is no doubt that a species has no existence in nature." This, however, is an academic question of general zoology, which should be treated by more competent hands than mine. In his rejection of von Lendenfeld's *Homodermidæ* and *Leucopsidæ* he has taken the only course open. It is to general advantage that it should be stated plainly that the histology figured in *Homoderma* is alone sufficient to convince any student of *Calcarea* that the structures described were never seen.

The attempt to group the *Homocœla* according to structure is valuable and suggestive. It does not claim to be sufficiently developed to be considered as a natural classification. There are three sections of the *Homocœla*: I. *Simplicia*, solitary, or with individuals of easily recognised individuality. II. *Reticula*, anastomosing tubes, in which individuals are unrecognisable. III. *Radiata* (one species), a (large) central Ascon-tube bearing secondary radial tubes. The last group or species (*Leucosolenia tripodifera*) is perhaps the most important discovery recorded in the paper. The radial tubes branch and branch again, until they are set thickly together like a wall round the wide central osculate sac into which they open, so as to simulate precisely the canal-system of a *Sycon*. But the central tube retains its collar-cells and pores, and its true wall differs in no structural respect from that of its tributaries.

This observation and its treatment by the author appear to me most suggestive. I think that an examination of intermediate forms will convince him that there are many other *Homocœla* to be included in the *Radiata*. *Ascandra*



*Lieberkuhnii*<sup>1</sup> without doubt, as it is found at Naples, comes under his definition of the group, and in its fir-tree-like form, branching at right angles into smaller and smaller tubes, shows a stage antecedent to *L. tripodifera*. Its main oscular tubes are as much (in the specimen before me) as 1.5 cm. by .4 cm.; its smallest branches about .01 cm.; between these all intermediate sizes. A most suggestive feature, on which this is not the place to dwell, is that the unpaired rays of the spicules in the branches are mostly distally directed. In *Ascaltis cerebrum* the oscula (not pseudoscule; Haeckel was probably misled by the outward appearance when he figured what Dendy would term an "inverted canal-system") open from spaces 0.3 cm. wide, lined with collared cells continuously up to the granular lip; its wide tributaries are not superficial as in the oscula of *Ascandra reticulum*, but deep, and covered with secondary ramifications, ranging down to about .01 cm. diameter. This sponge might seem almost to lead us to Dendy's *Leucosolenia stipitata*, placed by him in Section II. But without going further I would point out that the three previous forms all possess triradiates and quadriradiates more or less slender and pointed; that while those of *A. Lieberkuhnii* are of nearly the same dimensions as *L. tripodifera*, and the apical ray similarly curved, *A. cerebrum* possesses on its external surface the very tripods which give *L. tripodifera* its name. Will Mr. Dendy consider the possibility that his Radiata may already advantageously step forth as a genus? e.g. *Homocœla*. Genus 1. *Leucosolenia*. The growth of the tubes is mainly confined to new branches; type *L. clathrus*, *L. dubia*. Genus 2. *Nardoa*. The growth of the tubes is continuous, the newest branches have consequently smallest diameter; type *N. tripodifera*, *N. Lieberkuhnii* (?).

The Reticulata are again divided according as the gastral cavity is or is not traversed by ingrowths of mesoderm. I must state my strong suspicion that the "ingrowths of meso-

<sup>1</sup> For my own observations I use Haeckel's names to avoid the coining of new combinations.

derm " are the amœboid cells observed long since in the gastral cavity of certain sponges after digestion, which, as in *Ascetta clathrus*, form such traversing processes, and which I believe, with the older workers, to be collar-cells; to the amœboid metamorphosis of which Dendy makes no allusion. In these Australian sponges there appears to occur none with a many-layered endoderm. This structure, observed by Haeckel and since universally discredited, certainly appears in *Ascetta clathrus*, and—I hope I am not wrong in saying—was observed some years ago by Mr. Hardy, of Caius College, Cambridge, in a *Leucosolenia* found by him at Plymouth.

Turning to histology, Dendy finds "the ectoderm of the *Homocœla* agrees precisely with what Schulze has described for *Sycandra raphanus*." Although this form occurs in the *Homocœla*, it is in my experience rare. The typical ectoderm (e. g. *Ascetta clathrus*) I find composed of onion-shaped gland-cells containing a nucleus and granules, and provided with a usually fine duct, the expanded end of which forms the hexagonal area whose boundaries are, in the case of most sponges, all that has been observed. In *Ascetta clathrus* and *blanca* almost the whole ectoderm is of this type, and at least a large part of it in *Ascaltis cerebrum*, *Ascandra reticulum*, and *Ascetta primordialis*; on the external surface in *Sycandra raphanus*, *Leucandra aspera* (sensu Vosmaer), and a new sponge which I provisionally name *Sycaltis leuconides* (having a *Sycaltis* skeleton and a *Leucon*-like canal-system, and thereby necessitating a change of classification among the *Heterocœla*). Making such a statement without details or figures, I will add that in 1887 Dr. Vosmaer very kindly volunteered to me permission to quote him as being convinced with respect to the ectoderm of *Leucandra aspera*. This structure of ectoderm was described and figured by Merejkovsky for *Halisarca* in 1878 ('Mém. Acad. Petersburg'), by Metschnikoff for *Ascetta blanca* in 1879 ("Spong. Studien," 'Z. f. w. Z.,' 32). Though occurring in one of the latter's

best known papers I have never seen this description alluded to, and the entire paper has curiously escaped Dendy's notice. Metschnikoff's pl. xxi, fig. 1, gives evidence of the flask-shaped epithelium in the young *Halisarca*, corroborating Merejkovsky's description, with which, indeed, the figures of Schulze and similar ones of Metschnikoff's are at variance only in interpretation. I have found this glandular ectoderm in a horny sponge (*Aplysina* ?), an *Axinellid*, and a *Renierid*. Von Lendenfeld's "mesodermal gland-cells" are certainly nothing else in *Calcarea*, and as the descriptions are identical they are probably the same in horny sponges; many "æsthocytes" and similar structures in all likelihood have the same foundation in fact. I therefore personally believe that the typical ectoderm, not only of *Calcarea*, but of sponges, is a glandular epithelium of flask-shaped cells with dilated mouths, and that on external surfaces this is probably the usual form.<sup>1</sup> Mr. Denby has shared with most others the disadvantage of working on specimens more or less shrunk by preservation in alcohol; to this shrinking, to generalisation from the epithelium of canals (more easy to observe than the defended exterior), and to deduction of the existence of a flat epithelium from mere hexagonal silver lines, I attribute the overlooking of this structure by all but the two Russian authors.

Dendy has not found cilia on the ectoderm of *Homocœla*, and throws much doubt on the figures of Lendenfeld, where they are invariably introduced. After long comparison of Dr. von Lendenfeld's descriptions of *Calcarea* with the original structures I have no hesitation in saying that his "low flat plates filled only partially with protoplasm; from this plate threads extend which pervade the cell-cavity," are completely imaginary. His flagella certainly do not exist in the *Calcarea* I have examined; they are probably a generalisation from some structures he has seen in horny sponges. In *Aplysina* (?) I have found threads standing vertically from the ectoderm and precisely simulating flagella; they are undis-

<sup>1</sup> I think that it may prove the primitive Metazoon ectoderm, and will probably be found in various larvæ.

solved in 30 per cent. caustic ammonia, and are probably vegetable.

In the case of the sheath on the apical ray of gastral spicules, Dendy most justly says that there is no evidence to prove their mesodermal origin, and I differ from him in that I am thoroughly prepared to accept them as endoderm. The time has come to free the study of the Porifera from the fetish of mesoderm, and to render to her grasp only that to which embryology can prove her entitled. The presumption lies in favour of the old layers, the ectoderm and endoderm; the *onus probandi* is on the new-comer.

My own work has led me to regard the endoderm not only as multiform, but as most proteic. Dendy recognises it as "polymorphic," but this appears only to refer to the relative state of "retraction" of the collars and flagella. I agree with all his description and figures of these, both in this and preceding communications, and I have now myself observed, in the living *Sycandra raphanus*, the coincidence of flagella and Sollas's membrane which (in *Halichondria panicea*) he was the first to meet with. But I have come to the conclusion that this coincidence is only transitory; and while he most generously yields priority for the theory of filtration, I have been brought to relegate it to the disunited collars, and to believe that the membrane of Sollas is a valvular adaptation to prevent the reflux of water past satiate and therefore inactive cells. Where he writes "retraction" I would write "disappearance," and I believe that in the old "*Verwandlung der Geissel bewegung*" in "*Amoeboide Bewegung*" lies the key to many of the anomalies of the intimate structure of sponges. He draws and describes in *Leucosolenia cavata* "yellow granules," which he more than suggests are symbiotic algæ. I have worked at them—besides in former years—during the last nine months in *Ascetta clathrus* (where, besides the description he quotes from Bowerbank, they were described and figured by Metschnikoff, loc. cit.) and *A. primordialis*. I find Dendy's drawings and descriptions of their behaviour and relations most accurate; in *Asc. clathrus* there is an additional point of interest that the



granules in the (glandular) ectoderm-cells differ from these only in being of smaller size. I have been very slowly and gradually led to the conclusion that the bodies in question, which I propose to call "Metschnikoff cells," are metamorphosed collar-cells; that by their reaching to the exterior and becoming perforated, pores are formed; and that the granules of these and of the ectoderm, and of the glandular ectoderm in general (and possibly the granular cells so frequently described beneath it in *Silicea*), are excretory.

In the nucleus of the ovum Dendy finds in *L. pelliculata* nucleoli and circumferential granules; in *L. depressa*, in addition, a faint reticulum. In *Asc. clathrus* (nitric acid and borax carmine) I find a distinct and typical reticulum with small granules at the nodes. I have found also a large nucleolus with vacuoles, possibly artificial. In the matrix capsule of sponge embryos Dendy has almost established a proprietary interest; in *Leucosolenia Wilsoni* he finds they have no connection with ectodermal cells. In *A. clathrus* ova occur which appear to have such a connection, but when full of yolk they lie in sacs dependent in the gastral cavity, clothed with collared cells, of which some are always metamorphosed, and which are in continuity by the neck of the sac with the general endoderm. It may be worth adding to his instances of specially robust external spicules, besides *Asc. cerebrum*, whose "tripods" are confined to the outer surface, *Ascandra reticulum*, in some varieties of which the acerate ("orceote") spicules are so confined, while in others they disappear. The rod-like bodies he describes on the gastral surface of Sollas's membrane in *L. tripodifera* I do not believe to belong to the sponge; he himself accepts them doubtfully. But it is curious that in the allied *A. cerebrum* and the variety of *A. primordialis* which simulates its form (variety of *A. cerebrum* simulating the spicules of *A. primordialis*? nova species?) the collars and flagella most frequently appear to be replaced by a network of threads. Nothing but its "constancy and peculiar and regular arrangement," to quote Dendy's words, could, however, give any doubt that these are vegetable, which on the whole is at present

my belief. With reference to this membrane, the descriptions of von Lendenfeld, in contradiction to the statements of Sollas and Dendy, are, I have convinced myself, as worthless as those of the unique and quite different form of collar-cell till very recently described and figured by him in all groups of sponges.

Dendy's observations of afferent canals lined with ectoderm perforating the walls of *L. stolonifera* are most interesting, and in my judgment probably of generic importance. Though *A. clathrus* is much more thickly walled than most of the group, the communication is established by a single perforated granular cell as in other *Homocœla*, and strictly homologous with the granular ring round a prosopyle in the *Heterocœla* (cf. Poléjaeff, *Grantia tuberosa*). This description does not refer to other large pores which occur in this lipostomous sponge, whose structure and morphology I have not worked out.

In conclusion, if in this review points of difference rather than of agreement are accented, it is because the former take more words to express. This paper lies throughout in lines parallel to those on which I have been long labouring; and it may so not be impertinent to give it the cordial welcome of a fellow-worker who finds a great stride made forward towards the knowledge of a group that appeared almost insoluble.

GEORGE BIDDER.

NAPLES; August, 1891.

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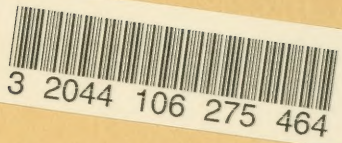
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